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FRANK J. ORLAND

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EDITOR'S VIEWPOINT

Having been duly elected to the Editorship by the I.A.D.R. membership and now installed as Editor of this worthy JOURNAL, I would like to indicate in a characteristically concise manner that I deem it a privilege and a challenge to serve in this capacity. The JOURNAL OF DENTAL RESEARCH already has a significant place among scientific publications but further effort on a collective basis should be exerted to make it a scholarly journal with the ability to publish with rapidity meritorious manuscripts which represent original investigative effort in the broad stomatological area.

Over the past twenty-two years, the outgoing Editor, Hamilton Burrows Greaves Robinson, has had a rather Gargantuan task and yet has done a commendable piece of work in carrying on the JOURNAL through both turbulent and complacent times. This present issue has been very completely edited by Dr. Robinson and hence his brief review of each paper under EDITOR'S VIEWPOINT appears as before, over his unique initials H. B. G. R.

—Frank J. Orland.

The study by Smith (p. 187), which won the first Edward H. Hatton Novice Award, demonstrates the active role of epithelium in separating normal from severely inflamed tissues and in eliminating fragments of bone and tooth from wounds. Not only is the presence of an "epitheliotaxin" suggested but one may ponder why such actively proliferating tissue ceases its invasion of connective tissue when its function is completed and why malignant tissue does not.

The application of the Papanicolaou technic for recognition of oral disease has interested a number of investigators. Silverman, Becks, and Farber (p. 195) found that the appearance of cells from benign oral lesions did not permit recognition of the different types of lesions. In 15 patients in whom malignancy was strongly suspected from clinical examination, 14 tests were positive by both biopsy and cytologic examination. In one instance, the biopsy was misleading and in another the cytologic finding was incorrect. In another group of 60 patients, 4 carcinomas were found by the Papanicolaou method. The "smear technic" suggests itself as a valuable screening mechanism and is worthy of evaluation in large groups such as are being reviewed for cervical cancer.

Luduena, Hoppe, Oyen, and Wessinger (p. 206), in animals anesthetized with pentobarbital, found levo-nordefrin to be 100 to 200 times more active than the dextro-isomer in raising blood pressure and that, in equipressor doses, levo-nordefrin increased heart pressure somewhat more than epinephrine. In equipressor doses epinephrine was more active than levo-nordefrin in stimulating the retractor penis, the levo-compound producing greater contractions than the dextro-compound. When tested on the uterus of nonpregnant rats levo-nordefrin was 5,000 more inhibitory than levo-nordefrin. The levo-compound appeared to be 10 times less toxic than the dextro- and to have ⅕ the toxicity of epinephrine. Levo-nordefrin significantly increased the duration of anesthesia. Since racemic nordefrin (Cobefrin) and levo-nordefrin (Neocobefrin) are being used in local anesthetics for dental purposes these studies are of immediate interest.

Fisher, Wait, Chalup, and Nash (p. 214) continued studies on the nitrogen content of dental pulps which has interested their laboratory for several years. They found no statistical difference in nitrogen content of pulps between species, and none between regions of the pulp, although they suggest that nitrogen content appears to increase slightly in more mature pulp tissues.

Seltzer (p. 220) studied pulpal changes in 10 teeth of one dog subjected to various operative procedures associated with crown preparations. Since the time elapsing between death of the animal and tooth preparation was only ½ to 2½ hours the changes seen microscopically were early. While hemorrhage, edema, and cellular infiltration would be expected in this time, one wonders

if "degenerative changes" in the tissues of the pulp were antemortem or post mortem. Preparation of good pulp sections is extremely difficult and artifacts or post-mortem changes are the rule rather than the exception. This study indicates that injudicious operative procedures may lead to pulp injury but the short time between treatment and cancellation did not give an opportunity to observe recovery or repair.

Berman and Massler (p. 229) investigated pulp repair in rat molars under zinc oxide and eugenol and calcium hydroxide. Regardless of medicament a "primary calcific bridge" formed in about 14 days. As might be expected, in a region of relatively fast calcification, this was poorly organized. At about 21 days a permanent bridge of tubular dentin appeared. The authors consider the primary bridge a product of "degenerating cells" and the permanent tubular bridge the result of "vital cellular processes." Vital processes, obviously, were involved in both and usually hastily calcified tissue is less regular in pattern than slower, more normally, calcified structure. Although the pattern of healing was the same at 21 days under either medication, the calcium hydroxide showed earlier superiority and this early advantage may be critical in the injured human pulp. It does not appear that calcium salts in the medicament are essential for repair.

Hall (p. 243) presents a very thorough report on electron microscopy of enamel. He observed small organic projections on the sides of the crystals, giving the crystals in the cervical region of newly erupted teeth an appearance like the edge of a postage stamp. About half of the organic material in the enamel fibers is replaced by inorganic material as the enamel becomes older. He suggests that the crystals become keyed to each other, preventing slipping.

Examining the tissues from teeth collected from cities with 0.1, 1.0, and 5.0 ppm fluorine in their water supplies, respectively, Isaac, Brudevold, Smith, and Gardner (p. 254) found that higher fluoride concentrations were present in the enamel surface than the inner enamel layers. At the lower levels (0.1 and 1.0 ppm fluoride) the fluoride content of the outer as well as the inner enamel increased with age, but at the level of 5.0 ppm the increase of fluoride with age was observed only in the inner enamel. With the fluoride content of water supplies increased, the amount of fluoride deposited in the enamel posteruptively had increased as well as that deposited during tooth formation. The authors confirm the observation that there is a direct relationship between the fluoride content of enamel and caries susceptibility. However, they point out that because there are many factors in addition to fluoride which influence the carious process the fluoride caries relationship is not a simple one and teeth of the same fluoride content do not necessarily show the same incidence of dental caries.

Using the same teeth they had used in earlier studies of fluoride content, Isaac, Brudevold, Smith, and Gardner (p. 318) investigated the solubility rate of subsurface enamel of teeth from cities with different fluoride content in the water supplies. Deeper layers of enamel were consistently more soluble than the outer layers; the greatest increase occurring between the first and second layers of enamel. While the fluorine concentration of successive layers decreased as the solubility increased within each group of teeth, enamels that were comparable in concentration of fluoride showed different solubilities. The differences in solubility between the surface and subsurface layers were shown not to be related to the organic content of enamel and there appeared to be no relationship between age and acid solubility of enamel. It is suggested that the accumulation of trace elements other than fluoride and other changes which may occur in enamel during its history must be considered in studying the resistance of enamel to decalcification by acids.

Using radioactive isotopes ($S^{35}O_4$) as a tracer in teeth of pigs fed diets very high in fluorides (1,000 ppm) and various staining methods, microincineration, microradiography, and phase contract microscopy, Bélanger, Visek, Lotz, and Comar (p. 264) investigated the development of the teeth. They concluded that dentin development is biphased with a stage of apposition and another of maturation. They consider the mucoid portion (probably mucopolysaccharide) of the ground substance of great importance in mineralization. In heavy fluorosed dentin, large spherules appeared to be calcium fluoride but deposits of white ash, seen in incinerated material, especially over the

calcio-traumatic lines, were not of calcium fluoride but might be chondroitin sulfate. The prolonged ingestion of very large doses of fluorides resulted in formation of deformed, immature and abnormally mineralized teeth in the pig.

Geller (p. 276) has re-analyzed the available data on collagen and on enamel and dentin proteins. On the basis of comparison with characteristics of the tooth proteins and of known keratins and proteins, he proposes that enamel proteins resemble collagen more closely than keratin. Since an entire theory of the caries process has been postulated on the premise that enamel proteins are keratins, this proposal is worthy of further investigation.

Stahl, Gerstner, and Joly (p. 280) injected somatotrophic hormone locally into the oral mucosa of young adult rats. The tissue was studied in histologic sections and in tissue culture. The treated mucosa showed increased acanthosis and keratinization of the epithelium, as well as an increase in connective tissue fibers. A striking feature was the increase of binucleate cells in the epithelium. When tissue from the same area was grown in tissue culture, migration of the epithelial cells occurred prior to migration of fibroblasts after explantation, a reversal of the usual order of activity. The epithelial cells appeared larger and closer than those seen in the control cultures and most of the epithelial cells from the experimental area had two or more nuclei. The somatotrophic hormone appears to have a local effect on the oral mucosa of the rat and this possible effect should be considered when somatotrophic hormones are being injected for treatment of diseased conditions in patients.

Relatively minor variations in technic may produce great differences in results. Boyd and Rosenthal (p. 288) examined material from gingival sulci and found bacteria present in 93 per cent of the cultures. This was in contradiction to a prior report that gingival sulci are sterile, but the authors who had reported sterility first had applied iodine and glycerine to the teeth and gingiva and then dried by airblast. From Boyd and Rosenthal's study, it appears that fluids from the oral cavity and microorganisms do enter the gingival sulcus.

Person, Wannamacher, and Fine (p. 292) deprived adult rats of protein and studied their oral and extraoral tissues, histologically, and for nitrogen content. Although the depleted animals and their organs were lighter, the per cent nitrogen content of tissues was the same except for liver which had less nitrogen and more fat. Osteoporosis was observed in the mandibles of all animals. The latter finding may have been related to the physical nature of the diet. The salivary glands and tongue showed regressive changes in the depleted animals.

Caldwell, Gilmore, Timberlake, Pigman, and Pigman (p. 301) have made microhardness tests of teeth subjected to in vitro caries. Among other findings was that fluorides can retard caries, in vitro. Moreover, as might be expected, fluorides gave greater protection to ground than to unground surfaces as the former would pick up more fluoride. This study, under the in vitro conditions that the authors choose to label the "artificial mouth," helps to demonstrate that lesions closely resembling, or even identical with, in vivo caries can be produced in vitro. It might be noted that in this JOURNAL the term "artificial mouth" has not been capitalized for such capitalization is usually reserved for registered trademarks which appear in some periodicals simply capitalized and in others with the designation ®. This "artificial mouth," as indicated by this paper, is a useful apparatus to produce in vitro carieslike lesions but others may more closely approximate the in vivo conditions in a special in vitro apparatus.

Rovelstad, Geller, and Cohen (p. 306) grouped 1,049 young men into 3 groups, 63 without evidence of past or present caries experience, 131 with 1 to 6 surfaces that had had caries, and 138 subjects selected at random. Although there were no significant differences between the groups in salivary pH, acid neutralizing ability, or hyaluronidase activity of saliva, the differences in flow rate, Snyder test, and lactobacillus counts were significant. It should be noted that these groups were all young naval recruits living under similar conditions and that the method of collection was regimented. In this way, many possible variants were eliminated or minimized. This may account for differences between this study and others that have failed to relate salivary bacterial indexes to past caries experience.

Anderson and Picton (p. 312) found the masticatory loads on lower first molars quite similar on the chewing side and the empty side during unilateral chewing. Raising the tooth surface level by 0.5 mm., only, increased the load by twice normal. Although this is a "traumatic" load it is not as great an increase as one might expect. Abnormal chewing patterns were produced by lowering the recording apparatus 0.5 mm. These physical studies may give clues to the variations in patterns of tissue response in "occlusal trauma." Since many individuals may be subjecting individual teeth to abnormal stress without showing evidence of periodontal disease, while others do have traumatic effects, the problem of physical loads is important as well as tissue reactions.

Buttner and Muhler (p. 326) found that placental transfer of fluorine in rats does not occur to a significant degree unless the mother consumes water at 10 ppm F or more. It appears that at higher concentrations some fluoride may be received through the milk. As the authors suggest, these data cannot be transferred directly to human subjects. It appears that in some individuals F placental transfer must be possible, for fluorosis is observed occasionally in deciduous teeth of children from high fluoride water areas.

Manhold (p. 330) presents a study of DMF (surfaces) vs Pensacola Z Scale scores. The Pensacola Z Scale score is designed to measure "personal autonomy and heteronomy." The results are suggested as indicating that greater tendency toward heteronomy (and the factors composing the syndrome) are related to a higher incidence of dental caries experience. While the results are suggestive, one must be extremely careful of mistaking casual relationships for casual causal relationships. Eighteen of the 22 references include the names of either, or both, Jones and Manhold, one emphasizing the "autonomous" personality and the other psychosomatic causes of oral disease.

Chilton and Fertig (p. 335) have outlined, in some detail, the construction of a clinical dental trial of a new caries inhibitory agent. They use the results of certain actual studies as examples. While the propriety of publishing such papers in a "research" journal may be questioned, the increasing interest in possible caries-inhibiting agents and the often apparent waste of time, money and, that rarer commodity, subjects appears to justify its publication and study.

Changes in inlay castings from the time the pattern is placed in the mold until the casting procedure takes place may account for undesirable dimensional change and distortion. Mumford and Phillips (p. 351) investigated these changes with 4 investing technics and with various positions of the pattern in the mold. Expansion was greater as the pattern was moved toward the crucible-former end of the ring, or toward the sides. All technics produced distortion—beginning 1 minute after initial set of the investment and reaching the maximum in 1 hour. Although our investment technics and materials have been fairly well standardized, there is still need for much better methods and materials if the inlay technic is to be as good as it might be.

Ryge (p. 359) utilized the principle of fluorescent x-ray spectroscopy to develop a method for mercury analysis of dental amalgams. The preparation of specimens is critical and the electronic equipment must be carefully controlled and standardized repeatedly. However, the method is rapid, specimens are not destroyed, and it is accurate except for analyses of mercury content in the 45 to 65 per cent range.

The electro-deposition of a thin layer of gold on austenitic steel, prior to soldering, has been recommended to ensure a corrosion-resistant joint. Accelerated crevice corrosion tests and microscopic study of sections of such joints studied by Bien and Ayers (p. 368) did not confirm the value of this procedure. Confirming clinical studies, they found 80 nickel-20 chromium much more resistant to crevice corrosion, especially when gold is flash-plated, than other rustless alloys. Since these alloys are being used widely for dentures and orthodontic appliances, knowledge of the relative resistance of various types of soldered joints is important.

The William J. Gies Foundation has announced a grant of $3,900 to the International Association for Dental Research to be used during the current year to increase the number of pages published in the JOURNAL OF DENTAL RESEARCH.

H. B. G. R.

THE ROLE OF EPITHELIUM IN THE HEALING OF EXPERIMENTAL EXTRACTION WOUNDS

ROBERT LEROY SMITH, D.D.S., M.Sc.

(College of Dentistry, Ohio State University, Columbus, Ohio)

THE fate of retained bone spicules and root fragments and the process of removal by the body are basic problems in the repair of extraction wounds.

The healing of extraction wounds has been studied in many experimental animals including rats,[2, 4] dogs,[1, 3, 6] and from human beings, including biopsy specimens[5, 7] and post-mortem tissues.[5] The majority of these studies have been primarily concerned with the time required for bony repair of the alveolar socket[1, 4, 6] and the time at which the epithelium covers the surface wound.[1, 2, 3, 5] The healing process with various therapeutic agents within the socket has been widely reported,[1, 3] often on only a clinical basis.

The healing process of extraction wounds, in the presence of retained bone and root fragments, has been reported.[2] The study included all structures of the socket, such as, periodontal membrane, blood clot, epithelium, alveolar bone and was concerned primarily with delayed healing in the presence of retained fragments. Mention was made of the interesting manner in which the epithelium formed cystlike extensions from the surface, which were apparently a means of removing particulate matter from the wound.

Review of the literature, however, failed to reveal a satisfactory explanation for the process involved in the elimination of bone and root fragments. This study was undertaken to investigate the mechanism of spicule and root exfoliation.

The results obtained suggested a relationship between retained bone and root fragments, inflammatory exudates, and an epithelial response to isolate and eliminate these foreign bodies from the wound. The consistency of epithelial response and its mode of proliferation suggested the presence of an "epitheliotaxin."

EXPERIMENTAL METHOD

The rat is a desirable animal for this type of study. The maxillary molars have 4 slender roots which are often hypercementosed and a thick, dense periodontal membrane, both of which make complete extraction difficult.

Presented at the General Meeting of the International Association of Dental Research at Atlantic City, N. J., on March 22, 1957. Awarded the Edward H. Hatton Novice Award for 1957.

This paper is based on a thesis presented in partial fulfillment of the requirements for the degree of Master of Science, Ohio State University, Columbus, Ohio. This study was supported in part by the U. S. Public Health Service, Grant No. D-168 (C-3).

Received for publication May 22, 1957.

Bilateral extraction of the maxillary first molars was carried out on 33 female Sherman strain albino rats ranging in weight from 200 to 250 Gm. The animals were anesthetized with 3 mg. of Nembutal intraperitoneally per 100 Gm. of body weight. The animals were then secured to a board to facilitate handling.

The extractions were accomplished by making a 2 mm. incision through the mucoperiosteum anterior to the teeth. A mucoperiosteal flap was elevated over the buccal and palatal aspect of the alveolus. The teeth were luxated with modified scalers which were used as elevators, and then removed with mosquito forceps. Multiple fractures of the roots and alveolar bone resulted from the elevating process. Following extraction no particular effort was made to débride the root and bone fragments from the wound. The animals were maintained on a stock Purina chow pellet diet and water ad libitum until sacrificed.

The animals were sacrificed at intervals of 1, 12, and 24 hours; 3 days; and weekly from 1 to 8 weeks. The specimens were immediately placed in a fixative, decalcified, sectioned, and stained in hematoxylin and eosin.

EXPERIMENTAL RESULTS

Microscopic examination was made of serial sections, at low and high magnifications, completely across the extraction wounds. All specimens were noted to contain sequestrating bone and root fragments. There was a remarkable similarity in the healing and repair process within a given group of animals at the arbitrarily chosen post-extraction periods.

The chronological sequence of the specimens provided a picture of the processes involved in repair.

One-Hour Specimens.—At this time the specimens show the alveolar bone with the clot-filled socket which contains fragments of retained bone and root. Remnants of the periodontal membrane and crevicular epithelium are present. There is a moderate edema of the mucoperiosteal flap which overlies small fragments of bone. There is no evidence of an inflammatory infiltration.

Twelve-Hour Specimens.—Similar socket contents are noted in these specimens, with a definite fibrin network within the clot. The edema of the mucoperiosteal flap is markedly increased and an inflammatory exudate is beginning to form. Accumulations of acute inflammatory cells surround retained bone and root fragments.

Twenty-Four Hour Specimens.—In addition to early organization of the clot, with the retained fragments, there is a definite response of the epithelium. These specimens show an epithelial proliferative activity at the fundus of the crevicular remnant and in the rete pegs adjacent to the wounds. The epithelium is noted to proliferate toward the areas of inflammatory exudate (Fig. 1).

Three-Day Specimens.—A proliferating rete peg has extended completely across the gingival papilla to unite with the crevicular remnant in an area of dense inflammatory exudate (Fig. 2). In some specimens these epithelial bridges presented areas of dyskeratosis.

One-Week Specimens.—Epithelial proliferation continues and, at this time, its role in isolating is first noted. The epithelium proliferates to separate vital

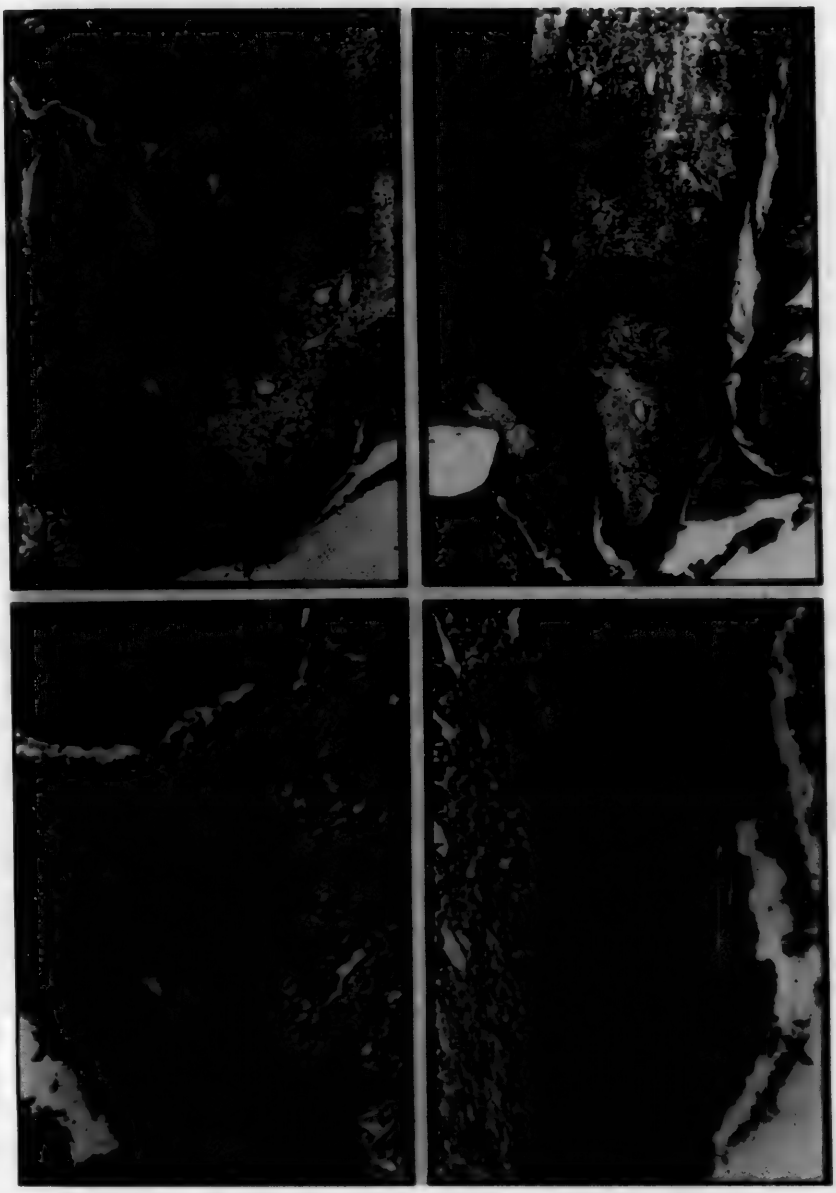

Fig. 1.—Twenty-four hour post-extraction specimen. The arrows identify the proliferating epithelium as it extends toward the retained bone and root fragments within the extraction wound (*X*) and toward the inflammatory exudate (*I*) over the buccal aspect of the buccal alveolar plate (*P*). Proliferation is also apparent in a rete peg adjacent to the crevicular epithelium. (Orig. mag. ×35.)

Fig. 2.—Three-day post-extraction specimen. A rete peg has extended, by epithelial proliferation, across the gingival papillae to unite with the crevicular epithelium in an area of dense inflammatory exudate (*I*). (Orig. mag. ×35.)

Fig. 3.—One-week post-extraction specimen. The proliferating epithelium is dividing the dense inflammatory exudate (*I*) from healthy connective tissue and progressing to isolate the retained root fragment. (Orig. mag. ×100.)

Fig. 4.—One-week post-extraction specimen. A dense layer of inflammatory exudate (*I*) separates the proliferating epithelium from the nonvital buccal alveolar plate (*P*). (Orig. mag. ×100.)

Figs. 5-8.—*For legend see opposite page.*

from nonvital tissues with its basilar surface always approximating healthy granulation tissue (Fig. 3). Nonvital calcified tissues are separated from the proliferating epithelium by a dense layer of inflammatory exudate (Fig. 4). The epithelium appears to be able to identify those areas of nonvital bone which will be sequestrated by resorption. Proliferation of epithelium progresses toward the areas of resorption which occur at the boundary between vital and nonvital bone, as defined by the presence or absence of osteocytes (Fig. 5).

Two-Week Specimens.—The isolating potential is fully demonstrated when, at 2 weeks, large areas of nonvital tissue are almost completely surrounded (Fig. 6). This function will be fulfilled as soon as resorption with sequestration of nonvital bone is completed.

Three-Week Specimens.—Complete isolation of nonvital tissue has taken place within this period (Fig. 7). Elimination of the debris depends only on the mechanical action of the tongue or food.

Four-Week Specimens.—Specimens in which there has been a low degree of inflammatory infiltration show a correspondingly low proliferative activity of the epithelium (Fig. 8). It appears that a dense inflammatory exudate is essential for complete isolation for, although proliferation occurs, the epithelium only approximates the fragments.

Six-Week Specimens.—These specimens again show complete isolation of fragments in animals which have had an adequate inflammatory exudate (Fig. 9). While those of low inflammatory infiltration show proliferation only to the point of proximity with the fragments (Fig. 10).

Eight-Week Specimens.—By this time the areas of nonvital tissue have been completely isolated and eliminated. The sockets are nearly filled with new bone and the mucosal surface is smooth. The only remnants of epithelial activity are the elongated rete pegs (Fig. 11).

DISCUSSION

The elimination of bone spicules and root remnants from extraction wounds has long been a problem to the practicing dentist. The results of this study permit a clearer understanding of the process by which the fragments reach the surface.

The epithelium was found to be the primary agent involved in the elimination of retained bone and root fragments. The process is accomplished by

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Fig. 5.—One-week post-extraction specimen. The epithelium has proliferated toward an area of bone resorption (*R*) which is the boundary between vital (*V*) and nonvital (*NV*) bone. (Orig. mag. ×35.)

Fig. 6.—Two-week post-extraction specimen. The epithelium has proliferated over the buccal and palatal aspect of the alveolus toward areas of bone resorption (*R*). The epithelium is also seen covering the granulation tissue in the base of the socket and separates it from a dense inflammatory exudate (*I*). Isolation of nonvital tissue and debris will be completed as soon as resorption of bone and epithelial union is completed. (Orig. mag. ×21.)

Fig. 7.—Three-week post-extraction specimen. The epithelium has completely isolated bone and root fragments, hair shafts, food, and other debris. (Orig. mag. ×21.)

Fig. 8.—Four-week post-extraction specimen. Epithelial proliferation is greatly retarded when the inflammatory exudate is lacking. The epithelium here appears to be attached to the retained root fragment. New bone is seen to be filling the alveolar socket. (Orig. mag. ×21.)

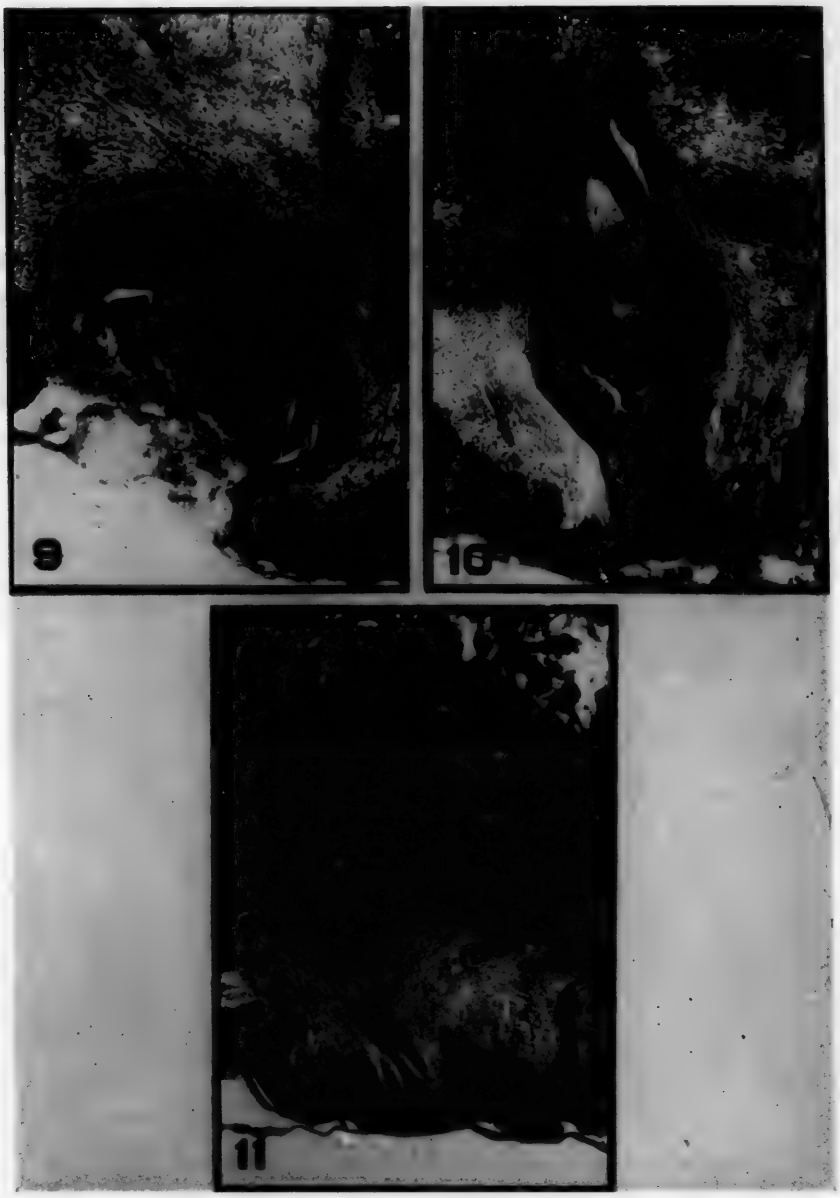

Fig. 9.—Six-week post-extraction specimen. The epithelium has completely isolated the retained root fragment which would have been eliminated soon by mechanical action of food or the tongue. (Orig. mag. ×35.)

Fig. 10.—Six-week post-extraction specimen. The epithelium is seen to approximate the retained root fragment in this specimen where a dense inflammatory exudate has not formed. A fistulous tract is noted from the root to the epithelial surface. (Orig. mag. ×35.)

Fig. 11.—Eight-week post-extraction specimen. Isolation and elimination has been completed. New bone has filled the alveolar socket and, with the exception of the elongated rete pegs, the epithelium is normal. (Orig. mag. ×21.)

epithelial proliferation around the retained fragments and exteriorizing them. This enveloping action proceeds from all margins of the alveolus and joins in the depths of the socket below the fragments and inflammatory exudate. This leaves a fistulous tract or opening to the mucosal surface. This opening remains patent until elimination of the non-vital tissue is completed. The epithelium consistently proliferates in the direction of areas of inflammatory infiltration, as if attracted to these areas. The extent to which the epithelium proliferated appears to be dependent upon and to be proportional to the concentration of the inflammatory infiltration.

This consistent relationship between inflammatory infiltration and the epithelial response suggests the presence of an epitheliotaxin, which is apparently liberated, in some manner, by the areas of inflammation. This study was entirely histologic and does not permit any more than conjecture as to the nature of this tropism. Whether it is enzymatic in nature, the result of local nutrition, an acid-base balance, or other phenomena cannot be determined from this study.

It was interesting to note that epithelial proliferation took place along a fragment of bone or tooth only if there was an interposed layer of inflammatory exudate between the epithelium and the fragments. In those specimens where an inflammatory exudate was lacking the epithelium became attached to either bone or cementum and the isolation process retarded. There was no resolution of areas of dense inflammatory exudate.

Several specimens, as early as 24 hours post-extraction, revealed a reversal of polarity in the rete pegs adjacent to the extraction wound. They were noted to extend completely across the gingival papillae where they would unite with the crevicular epithelium. This same process may aid in explaining the formation of the periodontal pocket. It has been known for many years that chronic gingivitis precedes the formation of the gingival pocket. If the rete pegs of the gingival epithelium would proliferate to isolate these areas of inflammatory infiltration in the crevicular connective tissue, and unite with epithelial rests in the periodontal membrane, they would be situated in a position to reattach to the cementum below the previous attachment. The inflammatory infiltration and nonvital tissue could then be eliminated and the gingival pocket increased in depth.

Some of the specimens in which a reversal of polarity occurred demonstrated areas of dyskeratosis and cystic formation in the center of the epithelial bridges. The ability of chronic inflammation to produce a reversal of polarity of the basal layers of the surface epithelium, with an ingrowth of the basal layers into healthy connective tissue, further suggests the theory of chronic irritation being an etiologic factor of epithelial neoplasms.

SUMMARY AND CONCLUSIONS

This study demonstrates the manner in which retained bone and root fragments are eliminated from experimental extraction wounds of albino rats. Study of the histologic sections suggest a relationship between retained bone and root

fragments, inflammatory exudates, and an epithelial response. The consistency of this epithelial response, to isolate and eliminate nonvital material, suggests the presence of an epitheliotaxin.

1. There is a relationship between retained bone and root fragments, inflammatory exudates, and an epithelial response.

2. The degree of epithelial response is proportional to the concentration of inflammatory infiltration.

3. The epithelium carries out an active process to isolate and eliminate bone and root fragments from the extraction wound.

4. The presence of an epitheliotaxin is suggested.

The author wishes to express his appreciation to the members of the faculty and especially to Dr. William Lefkowitz for guidance and assistance throughout the course of this study. Appreciation is also acknowledged to Mrs. Vaughn Miller, Mrs. Melvin Payne, and Mrs. Donald Swales for their technical assistance.

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THE DIAGNOSTIC VALUE OF INTRAORAL CYTOLOGY

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THE problem of oral diagnosis often becomes complicated by the lack of applicable and simplified laboratory tests. The microscopic tissue examination still remains one of the most useful procedures but is frequently delayed or omitted. The introduction of the sponge biopsy technic for intraoral lesions[4] was an attempt to reduce some of the disadvantages of tissue biopsy. Consideration was given to the possibility that a study of individual cells might aid in the diagnosis of intraoral lesions.[5, 6, 7, 8] The cytologic technic obviates many of the undesirable features of a biopsy and offers a method of great practicability. Its application depends upon a thorough familiarity with appearances of the cells from the superficial oral mucosa in health and disease. If reliability of differences in single cell patterns could be established, cytologic studies might possibly alleviate the existing breach between clinical impression and definitive treatment. The intent lies not in the choice of biopsy or cytology, but of cytology in preference to nothing.

Papanicolaou[11] noticed cells which he could definitely identify as abnormal, if not malignant. Papanicolaou and Traut[12] published a monograph on the *Diagnosis of Uterine Cancer by the Vaginal Smear* that established the cytologic technic as an effective diagnostic procedure and became the stimulus for widespread cytologic investigations. Cytology had opened the doors to many heretofore inaccessible areas[2] for diagnosis and is now accepted as a reliable procedure wherever cells may be obtained and studied.[1]

METHOD

The suspected area is first dried with a cotton sponge or cotton-tipped applicator to minimize contamination by cells shed from other areas. The best results have been obtained by collecting the cellular material with a slight rolling motion by a cotton applicator moistened with physiologic saline solution. The material thus obtained is then spread rapidly upon a clean glass slide which is placed immediately into a fixative consisting of equal parts of ether and 95 per cent ethyl alcohol. When the material obtained is scanty, the smear is confined to a small area of the slide to avoid drying in thin areas. A moment's delay in placing the slide into the fixative after its preparation will result in marked cellular deterioration due to drying and, although speed is

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essential, care must be taken to place the slide into the fixative gently to avoid dislodging the cells. Smeared slides are left in the fixative indefinitely, or they may be stained after a minimum fixation period of ½ hour. (If smears are to be referred elsewhere for staining and examination, they can be transported in the fixative unless they are mailed. Since postal regulations forbid mailing inflammable solutions, the slides can be air dried, following minimum fixation, and packaged.) When the smears have been properly prepared and fixed, drying after fixation does not significantly alter cellular detail when stained.

The Pharr modification of the Papanicolaou method[13] utilizing hematoxylin, Orange G and eosin is used in staining. This method affords consistency of staining reaction and excellent differentiation of cellular detail. Approximately 1½ hours is required to complete the procedure from the collection of the smear to fixation, staining, and microscopic examination. A single direct smear from an intraoral lesion affords adequate cellular representation of its cytologic changes and is preferred to multiple scant smears from the same lesion.

NORMAL INTRAORAL CYTOLOGY

Previous studies of exfoliated cells from the normal oral mucosa have emphasized the differentiation of squamous epithelial cells based upon cytoplasmic staining reaction.[5, 6, 15] Findings by these authors met with appreciable agreement. The following study is to be used as a base line for an investigation of cytologic findings in a variety of pathologic lesions of the mouth.

Smears were prepared from clinically normal buccal mucosa, hard palate, dorsal surface of the tongue, gingiva, and the floor of the mouth from males and females in different age groups. They consisted predominantly of squamous cells (Fig. 1) which line the oral cavity, with occasional amorphous matter, bacteria, and leukocytes. Small groups of columnar cells were an infrequent finding and were thought to originate from contaminating nasal or pulmonary secretions. Differential counts of the relative numbers of cornified, precornified, and basal squamous cells were tabulated. Sufficient nuclear and cytoplasmic variations were observed, not only from one patient to another, but also within a single smear, to preclude significant counts. This inconsistency may have resulted from both local as well as systemic factors which constantly alter cellular function and characteristics.

In spite of the inconsistencies, a relationship appeared to exist between cytoplasmic cornification and local functional irritation, a finding which is in general accord with results reported by others.[5, 6, 10, 15] The degree of cornification was determined by cellular morphology in addition to cytoplasmic staining reaction. The immature basal cells are recognized as spherical, epithelial cells with centrally placed nuclei which are consistently basophilic and thus the cytoplasm is stained green or blue-green by the Papanicolaou procedure. In the process of maturation, squamous epithelial cells flatten, the cytoplasm becomes progressively transparent and the nucleus contracts to a small pyknotic mass of chromatin. Cornified cytoplasm is acidophilic and may stain pink, yellow, or orange in a nonspecific fashion. Cells of intermediate

maturity are termed precornified cells and are recognized as squamous epithelial cells which have flattened slightly and show some degree of nuclear contraction. Precornified cells are usually basophilic, although cellular morphology is more reliable as a criterion of maturity than staining reaction.

No cellular or nuclear abnormalities were observed in the smears obtained from the series of patients who were considered to have normal intraoral mucosa. Smears obtained from the palate showed the highest degree of cornification and many of the squamous cells from this area were anucleated. Smears taken from the floor of the mouth displayed epithelial cells of the least maturity and smears from the gingiva, tongue, and buccal mucosa shed squamous cells of intermediate maturity.

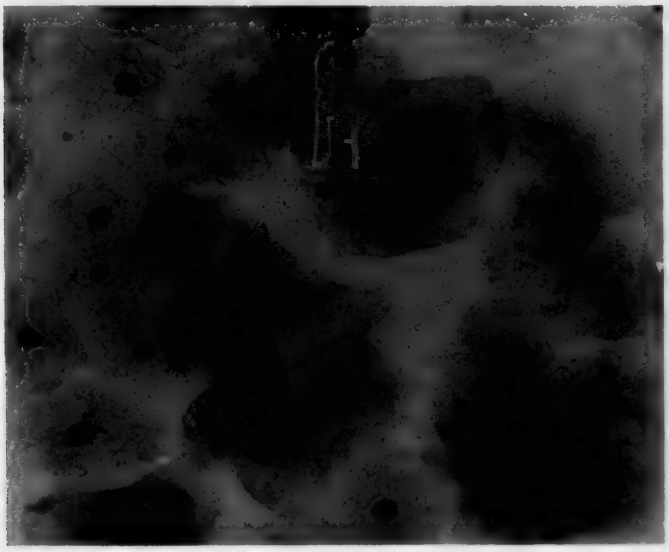

Fig. 1.—Squamous cells smeared from clinically normal buccal mucosa. There is uniformity in size and shape of the nuclei, which also display even chromatin distribution. Surrounding cytoplasm is uniform and abundant (small nuclear-cytoplasmic ratio). Staining reaction of the cytoplasm was approximately half acidophilic and half basophilic.*

*Due to the prohibitive expense of colored plates, the cytologic smears are reproduced in black and white, which unfortunately prevents the visualization of the characteristic staining reaction of the cytoplasm.

EXFOLIATED CELLS FROM BENIGN INTRAORAL LESIONS

Smears were obtained from known benign oral lesions to provide material for the second study group. Initially, it was hoped that it might be possible to classify these lesions on the basis of observed cellular morphology. However, differences from the normal group were too slight to permit an identification of specific nonmalignant disease entities. In general, lesions due to chronic irritation shed predominately mature squamous cells, whereas smears taken from areas of deeper ulceration contained an increased number of immature basal cells. Nuclear changes observed in the benign intraoral lesion group were limited to an occasional slight enlargement and, in a few instances, moderate hyperchromatism.

Fig. 2 illustrates a lesion from a 44-year-old woman who complained of a loose right lower incisor with surrounding ulceration. Even though the tooth was subsequently extracted, the ulceration increased in size. Three weeks postoperatively, clinical examination revealed a fungating lesion with irregularly raised and slightly indurated borders. Radiographs were negative and there was no lymphadenopathy. The differential diagnosis included granulomatous lesion secondary to bacterial or fungal infection or epithelioma. Direct

Fig. 2.

Fig. 3.

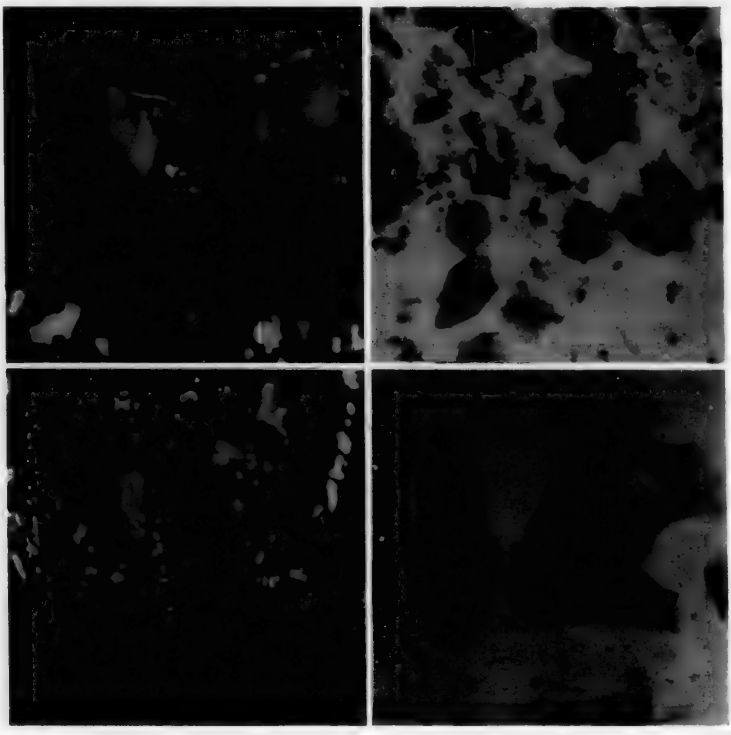

Fig. 4.

Fig. 5.

cytologic smears from the lesion contained normal squamous epithelial cells and abundant leukocytes (Fig. 3). No cells deviating from the normal were observed; however, the lesion did not respond to any chemotherapeutic or antibiotic agents used. A block surgical removal was performed. Serial sections revealed a subacute nonspecific inflammatory process. Other stainings and cultures added no more clarity to the diagnosis.

Fig. 4 shows a glosseal lesion in a 52-year-old woman with a history of glossitis of approximately 20 years' duration and who reported recent pain in one area. The lesion, which had been asymptomatic until this time, included an area in which fissures and slight ulcerations were seen. No lymph nodes were palpable. Representative cytologic smears taken from the break in the

epithelium contained only normal squamous cells (Fig. 5). Conservative surgical and fulguration procedures and biopsy confirmed the benign nature of this lesion.

In the 2 cases cited no cellular alterations were seen in smears from benign lesions of the mouth which would distinguish them from smears from normal mucous membrane. This occurs in contrast to other sites, such as the respiratory and female genital tract, where exfoliated cells shed in nonmalignant diseases are often markedly altered and may sometimes closely simulate malignant change.[3]

EXFOLIATED CELLS FROM MALIGNANT INTRAORAL LESIONS

Previous studies[7, 9, 14] have indicated the value of the smear technic for the early detection of intraoral carcinoma. Additional studies are indicated to evaluate the full potentiality and clinical usefulness of this procedure in dental practice.

Direct cytologic smears were obtained from proved cases of intraoral squamous-cell carcinomas to study the morphologic range of cells shed from this lesion. The malignant cells observed in this series conformed to the cytologic criteria of malignancy which has been established for the recognition of carcinoma of the cervix, bronchus, bladder, etc. These features include: (a) enlarged nuclei, (b) variation in nuclear size and shape, (c) increased nuclear/cytoplasm ratio, (d) multiple and prominent nucleoli, (e) hyperchromatism, (f) abnormal chromatin pattern and distribution, and (g) discrepancy in maturation in groups of malignant cells. In well-differentiated squamous carcinoma, the malignant cells contain hyperkeratinized cytoplasm and are often seen in bizarre shapes, such as the "tadpole" and "spindle" forms. The characteristics of the cells conformed well with established criteria for malignancy in other parts of the body.[3, 11, 12]

A series of 75 patients with ulcerations of undisclosed etiology were studied by biopsy procedure and the cytologic smear method. These were divided into 2 clinical groups. The first group included 15 patients in which carcinoma was strongly suspected and immediate biopsy was indicated. The second group consisted of 60 patients with complex ulcerations in which there was no definite indication for biopsy.

Cytologic smears preceded biopsy in all patients in the first group. Fourteen of the cases were reported to be malignant by both cytology and histopathology. One of the 15 cases was reported as negative by the cytologic method and one by the routine biopsy method. The negative results were in error by both cytology and biopsy. The false negative in the cytology group occurred in a case where microscopic examination of the tissue sections showed that normal squamous epithelium had proliferated over an ulceration of the tongue in which the tumor had invaded the connective tissue toward the tongue musculature. The false negative in the biopsy series was apparently caused by obtaining a tissue specimen from an area which was not representative of the malignant activity of the tumor.

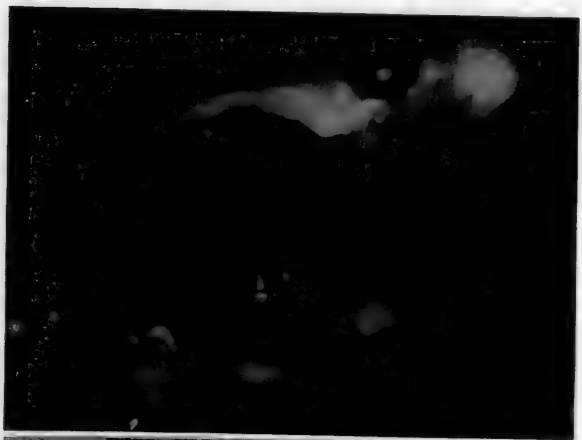

Fig. 6.

Fig. 7.

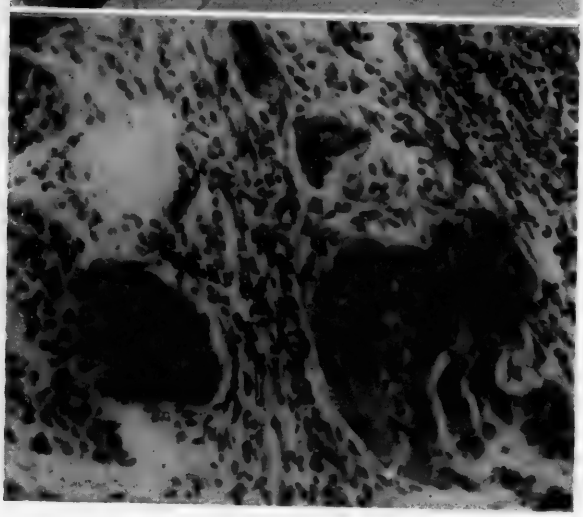

Fig. 8.

Each of the second group of 60 patients was initially studied by cytology alone, since biopsy was not thought to be indicated. Smears from 4 of the 60 patients were reported by cytology to be "consistent with malignancy" and a diagnosis of carcinoma was subsequently established by biopsy in each case. There were no false positive or false negative cytologic reports in the series of 60 patients. The detection of 4 unsuspected oral carcinomas in the group of 60 patients demonstrates the value of cytology in evaluating lesions which do not present convincing signs and symptoms of malignancy, particularly when biopsy does not appear to be indicated, or is not performed. The critical importance of early diagnosis of intraoral carcinoma emphasizes the value of the smear technic as a simple and accurate procedure for cancer detection and the incidence of false positive cytologic diagnoses of malignancy can be reduced to a rare occurrence through training and experience.

Unfortunately, suspicious areas of intraoral leukoplakia, prior to fissuring or ulceration, do not lend themselves readily to the cytologic smear method, since only superficial, cornified cells are obtained in smears taken from these white, patchy areas. Although this lesion may be of a premalignant nature, it remains a diagnostic enigma prior to histopathologic examination, a finding also shown by Montgomery and von Haam.[8]

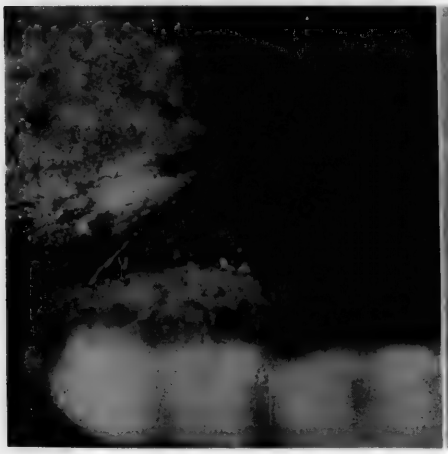

Fig. 9.

Fig. 10.

A 52-year-old white woman complained of a small, painful ulcer under her lower denture. In spite of denture relief, antibiotics and caustics, the ulceration progressed rapidly to involve a major portion of the floor of the mouth (Fig. 6). A smear taken from the lesion revealed cells which were consistent with malignancy (Fig. 7), and represented many of the classical criteria. Histologic sections from the surgical specimen revealed a squamous carcinoma (Fig. 8). A smear taken at the first appearance of this lesion might have hastened histologic confirmation and precluded the necessity for a commando procedure with a questionable prognosis.

A 46-year-old white man developed a lesion of the soft palate which was diagnosed as malignant (lymphoepithelioma) by biopsy. Following irradiation treatment, all signs and symptoms quickly disappeared, until a few months later a craterlike ulceration developed which contained necrotic material (Fig. 9). Clinical impressions varied between recurrence and radiation reaction.

Fig. 11.

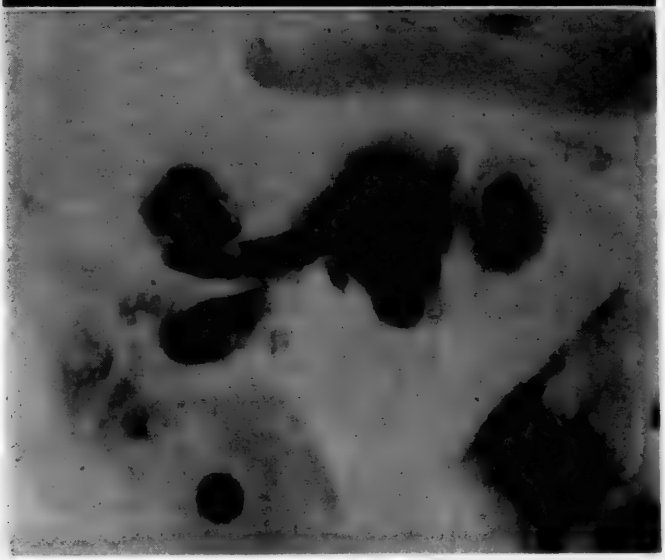

Fig. 12.

To biopsy the area presented a risk because of possible sequelae from surgery in an irradiated area, especially since the signs and symptoms were not clearly defined. Even though the cytology report (Fig. 10) was inconclusive, the

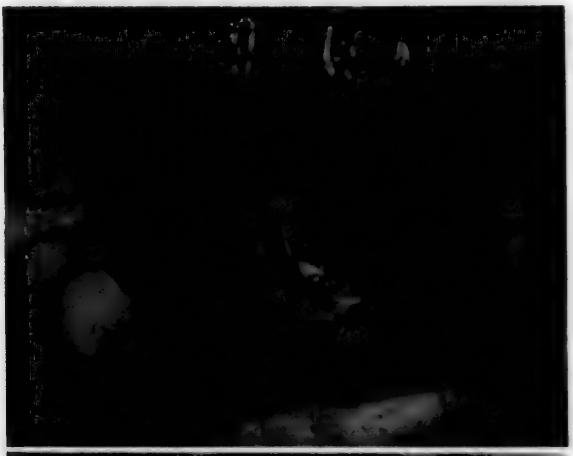

Fig. 13.

Fig. 14.

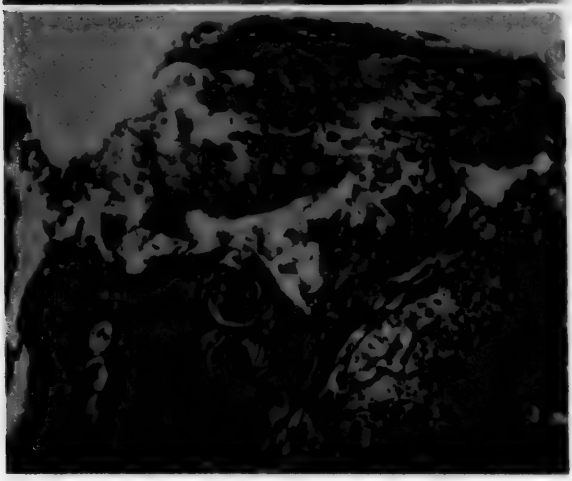

Fig. 15.

possibility of a malignancy was not ruled out. Repeat smears, entailing no complications, were taken and revealed cells which were consistent with malignancy.

A 65-year-old white man saw his dentist because of a loose and painful lower right second incisor, which was removed. On his first postoperative visit, an ulceration (Fig. 11, *a*), lingual to the extraction site on the floor of the mouth, was noticed. The clinical impression was that of an ulceration caused by local injury. A routine smear (Fig. 12) taken from the lesion contained numerous malignant cells. The patient, therefore, underwent immediate surgery.

A 48-year-old white man, who was a heavy smoker, was referred because of a lesion suspected to be a carcinoma of the floor of the mouth. The lesion was ulcerated (Fig. 13, *a*) and was surrounded by an area of leukoplakia (Fig. 13, *b*). A biopsy was reported to show no abnormality. Cytologic smears, however, confirmed the clinical impression of malignancy, displaying cellular features of clumping, pleomorphism, hyperchromatism, enlarged nuclei, and abnormal nuclear clearing (Fig. 14). The surgical specimen revealed squamous-cell carcinoma (Fig. 15).

SUMMARY AND CONCLUSIONS

Intraoral cytologic studies were undertaken utilizing a modification of the Papanicolaou procedure to evaluate the usefulness and diagnostic accuracy of the method in oral diseases. A base line for exfoliated cells shed from normal oral mucosa was established, and results indicate that each area of the mouth examined sheds squamous cells which are uniformly representative. A combination of cellular morphology and staining reaction was used to establish the relative maturity of normal squamous epithelial cells.

In benign oral lesions, cellular appearances did not show appreciable or consistent differences from the normal, to permit recognition or classification of the lesion. While it is often necessary to follow the clinical course of many benign entities, the critical importance of differentiating malignant from nonmalignant lesions indicates the need for a simple and reliable procedure for cancer detection.

Exfoliative cytology offers an excellent adjunct to biopsy for the detection of cancer because of its simplicity and accuracy. Its use is strongly urged as a routine procedure for lesions of unknown etiology when biopsy is delayed. It may prove valuable in suspected cases in which biopsy has failed to demonstrate a malignancy, and the two procedures can effectively be used simultaneously. Cytology can also be used in following treated oral malignancies to evaluate the effectiveness of therapy.

We desire to express our appreciation to Mr. S. Pharr for his technical assistance in the detail work of these studies, and to Miss Arlis Wilson, who performed most of the screening of the cytologic slides.

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SOME PHARMACOLOGIC PROPERTIES OF LEVO- AND DEXTRO-NORDEFRIN

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MANY investigators have studied one or more of the pharmacologic properties of racemic nordefrin* (2,4-dihydroxy-phenyl-3-hydroxy-2-isopropylamine) but much less is known about the differences in activity between the levo-† and dextro-isomers. Schaumann[1] has reported on the toxicity of levo- and dextro-nordefrin and their effect on the blood pressure of dogs and cats and the blood sugar and uterus of rabbits.

Studies in this laboratory[2, 3] have shown that the activity ratios of the optical isomers of isoproterenol and hyoscyamine were much larger than those indicated in the literature. These higher ratios were the result of a higher degree of purity of the dextro compounds. In view of these results, it was decided to study some of the pharmacologic properties of levo- and dextro-nordefrin, especially their relative activity. The resolution of nordefrin and the purification of the isomers by repeated crystallizations have been carried out and the results obtained with these samples on the perfused guinea pig lung have been reported recently.[4]

METHODS

Blood Pressure, Heart Rate, and Retractor Penis.—Mongrel dogs were anesthetized with sodium pentobarbital (32 mg./Kg.), intravenously. The carotid blood pressure was recorded with a mercury manometer and the heart rate with a Thorp impulse counter[5] adapted for this purpose.[6] Motility of the retractor penis was recorded by means of an isotonic lever connected by a thread to the muscle prepared by a technic which has already been reported.[7] The innervation of the muscle was intact. Vagal activity was inhibited by the subcutaneous injection at the beginning of the experiment of 0.5 mg./Kg. of a parasympatholytic drug, penthienate bromide‡ [2-diethyl-amino-ethyl-2-cyclopentyl (2-thienyl) hydroxyacetate methobromide]. L-epinephrine bitartrate, L-nordefrin base (M.P. 211.8°-212.2° C.) and D-nordefrin D-bitartrate dihydrate (M.P. 90.0°-92.2° C.) were used in our experiments. They were injected into the femoral vein. Doses were expressed in terms of the base.

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*Cobefrin, Cook-Waite Laboratories, Inc.

†Neo-Cobefrin, Cook-Waite Laboratories, Inc.

‡Monodral, Winthrop Laboratories.

Rat Uterus.—The effect of the sympathomimetic amines on the uterus of the nonpregnant rat uterus was tested in vitro by the method of Garcia de Jalon, Bayo, and Garcia de Jalon.[8] The percentile inhibition of the carbamylcholine chloride induced contraction, converted to probit, was plotted against the log of the concentration. Each of the concentrations graded at 0.3 log interval was tested on 8 to 10 uterine strips. The Effective Dose-50 (ED_{50}) was estimated from the curve. The values for levo- and dextro-nordefrin were obtained on the same strips. The stock solutions of the sympathomimetic amines were made in a solution of 1:100,000 of ascorbic acid and kept at low temperature. Dilutions in cold ascorbic acid solution were prepared a few minutes before addition to the bath.

Toxicity.—The acute intravenous toxicities of levo- and dextro-nordefrin were determined in white mice by the procedure described earlier.[9]

Local Anesthetic Potentiation.—Like other vasoconstrictors, levo-nordefrin increases the potency and the duration of anesthesia produced by local anesthetics. Two types of experiments were carried out: (a) the Threshold Anesthetic Concentration-5 (TAD_5) was determined in parallel tests using 3 dilutions of the original solutions graded at 0.15 log interval and 16 guinea pigs per dilution. The method of Bülbring and Wajda[10] was employed. The TAD_5 values were estimated from the dose-response curve obtained by plotting the average anesthetic score against the log of the concentration. (b) In the second test, undiluted solutions were tested in parallel to determine the duration of anesthesia. Readings were taken every 10 minutes until the average score for the wheal was below the 50 per cent value.

RESULTS AND DISCUSSION

In dogs anesthetized with pentobarbital, levo- and dextro-nordefrin produced, like epinephrine, a rise of blood pressure, cardiac acceleration, and contraction of the retractor penis. The change in the blood pressure tracing produced by levo-nordefrin (1 to 5 mcg./Kg.) was similar to that of epinephrine (0.5 to 2 mcg./Kg.). After a rapid rise, the pressure reached the initial level in 2 to 2.5 minutes (for rises up to 100 mm. Hg). Sometimes the pressure fell after the rise below the initial level, the changes in the kymogram being similar to those induced by epinephrine. Dextro-nordefrin, on the other hand, produced a more prolonged effect, as was noted by Schaumann.[1] The rise in pressure was not so rapid and there was no secondary fall.

After preliminary tests, 7 dogs were used to determine the relative pressor activity of epinephrine and levo- and dextro-nordefrin. Due to the limited number of animals tested and the fact that the epinephrin/levo-nordefrin activity ratio appeared to decrease as the experiment progressed only approximate values were obtained. Rises were matched side by side, within narrow dosage ranges.

Epinephrine was found to be 2 to 2.5 times as active as levo-nordefrin in raising the blood pressure. These values were obtained within the first 2 to 3 hours.

Levo-nordefrin was found 100 to 200 times more active than the dextro-isomer, on the blood pressure. Doses of dextro-nordefrin produced slight to moderate potentiation of the effect of the levo-isomer and of epinephrine.

No attempt was made to measure accurately relative chronotropic activity. In equipressor doses, levo-nordefrin was somewhat more active than epinephrine in increasing the heart rate. This is in agreement with results obtained with racemic nordefrin on dog's heart-lung preparations by Crismon and Tainter.[11]

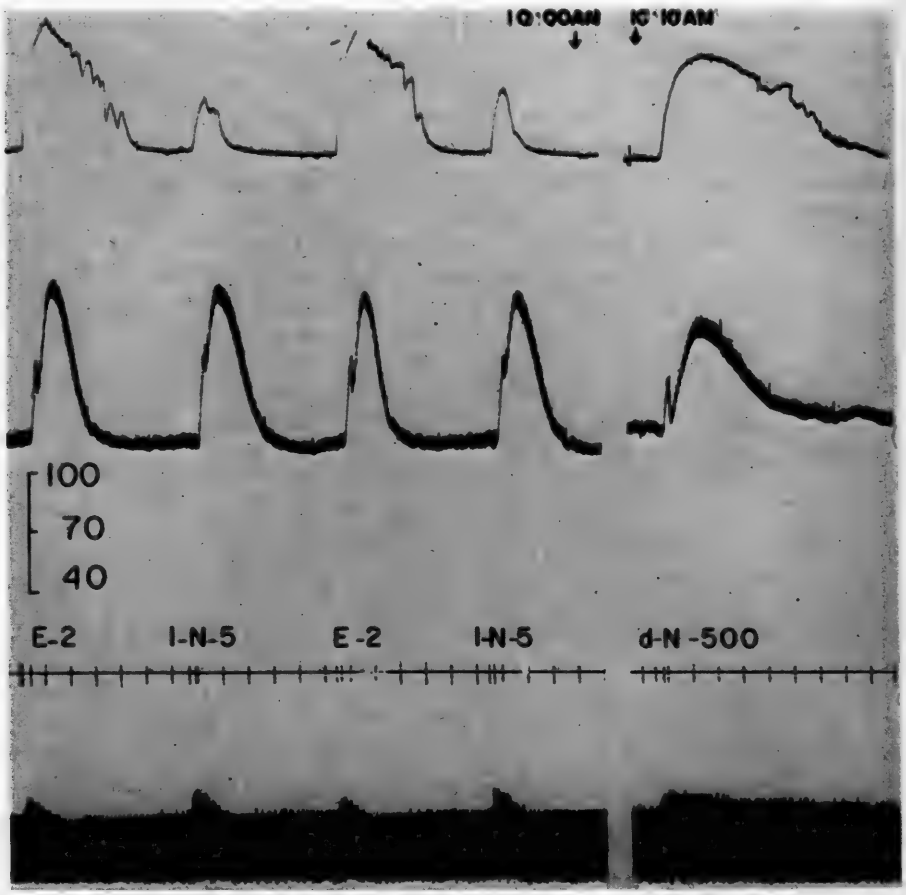

Fig. 1.—Dog, pentobarbital anesthesia. Tracings (downward): (1) Retractor penis; (2) Mean blood pressure; (3) Time in minutes and injection marks; (4) Heart rate.

E = epinephrine, l-N = levo-nordefrin, d-N = dextro-nordefrin. Number following letters indicates the dose in microgram per kilogram. Tracings show the effect of equipressor doses of epinephrine and levo-nordefrin and the effect of a dose of dextro-nordefrin 100 times larger than that of the levo-isomer.

Although levo-nordefrin was more active than the dextro compound on the retractor penis, stronger contractions were produced by the latter when equipressor doses were compared (Fig. 1).

Both levo- and dextro-nordefrin inhibited the rat uterus, as shown by their antagonism to the excitatory effect of carbamylcholine. Evident inhibition was obtained with concentrations of levo-nordefrin as low as 0.00125 mcg./ml. (1:800 million) (Fig. 2).

The ED_{50} values for epinephrine, levo- and dextro-nordefrin in micrograms per milliliter were as follows: 0.00021, 0.0032 and 17, respectively (Fig. 2). Epinephrine is therefore approximately 15 times more active than levo-nordefrin.

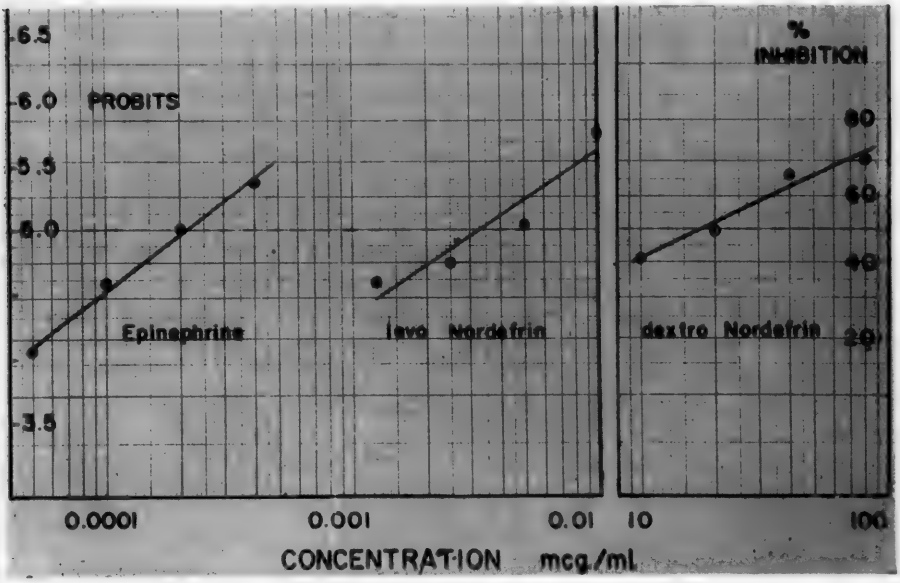

Fig. 2.—Rat uterus in vitro (method of Garcia de Jalon and co-workers). Percentile uterine inhibition plotted against concentration on probit-log paper.

Acute Toxicity.—The results are summarized in Table I. Similar data obtained earlier with epinephrine[7] are included for comparison.

TABLE I
INTRAVENOUS TOXICITY IN WHITE MICE

COMPOUND	NUMBER OF MICE	LD_{50} ± S.E. EXPRESSED IN MG./KG. OF BASE		APPROXIMATE TOXICITY RATIO AT 24 HOURS D-NORDEFRIN = 1
		24 HR.	7 DAYS	
D-nordefrin	50	128 ± 13	128 ± 13	1
L-nordefrin	40	12.6 ± 2.7	10 ± 4	10
Epinephrine	130	2.7 ± 0.2	2.5 ± 0.2	47

As shown by comparison of the 24-hr. and 7-day LD_{50} values, only in the case of dextro-nordefrin was the value the same. This and the fact that the activity ratio is 10 to 20 times larger than the toxicity ratio suggests that the mechanism of death is not identical for the isomers of nordefrin. However, sympathomimetic activity appears to play a part in the toxicity of the isomers.

The results obtained on anesthetized dogs agree with those obtained by Schaumann with the isomers of nordefrin. He pointed out that the differences between these compounds were also qualitative, as shown by the fact that cocaine potentiated the pressor effect of the levo-isomer and reduced that of the dextro compound.

The long duration of effect of dextro-nordefrin when compared with the levo-isomer on the blood pressure and the retractor penis could be attributed in part to a slower inactivation of the much larger but equipressor dose of the dextro-isomer.

The isomers differ also in their sympathomimetic activity pattern. In comparison with its enantiomorph, levo-nordefrin is much more active in producing inhibition of the rat uterus and the smooth muscles of the guinea pig bronchioles than on the blood pressure or the retractor penis of the dog. In fact when equipressor doses are tested the dextro-isomer is more active on the retractor penis. This difference in pattern may be based on the fact that the steric differences involve 2 asymmetric carbons.

Studies carried out in this laboratory have shown that, in regard to the pattern of sympathomimetic activity, the dextro-isomers of epinephrine, arterenol, and isoproterenol are not significantly different from the corresponding enantiomorphs. In other words, the levo/dextro activity ratios were approximately the same whether tested on the blood pressure, the rat uterus, or the guinea pig bronchioles. The relative position of the chain OH— group appears to change the activity of the compound in the same proportion on these test objects. This is not the case with levo- and dextro-nordefrin.

In the experiments designed to determine local anesthetic potentiation, 3 solutions were employed as follows:

Solution A		*Solution B*		*Solution C*	
Propoxycaine HCl*	0.4%	Propoxycaine HCl	0.4%	Propoxycaine HCl	0.4%
Procaine HCl†	2.0%	Procaine HCl	2.0%	Procaine HCl	2.0%
		DL-Nordefrin	1:10,000	L-Nordefrin	1:20,000

The actual TAD_5 values obtained varied somewhat from one test to the other as might be expected but the ratio for the 3 solutions was almost exactly 1:2:2, respectively. Although the addition of levo-nordefrin to the anesthetic solution doubled the potency as measured by this method, there was no significant difference between the solutions containing 1:10,000 of the racemic and 1:20,000 of the levo form of the compound.

In the tests designed to measure duration of anesthesia, undiluted solutions were run in parallel and readings were taken every 10 minutes until the average score for the wheal was below the 50 per cent value. The result is shown graphically in Fig. 3. With the solution containing levo-nordefrin 1:20,000, the average anesthetic score reached the 50 per cent value (score ±3) in 164 minutes. The duration was 6 per cent shorter than with the solution containing racemic nordefrin 1:10,000. Another test run as a check gave approximately the same result.

*Ravocaine, Cook-Waite Laboratories, Inc.
†Novocain, Winthrop Laboratories.

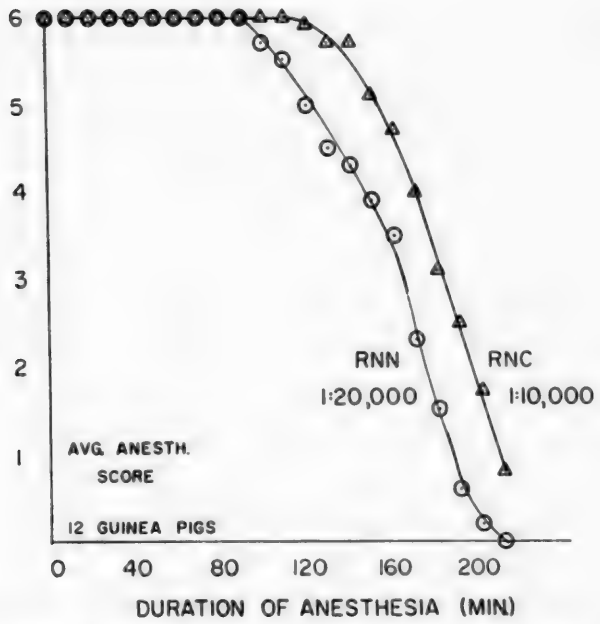

Fig. 3.—Intradermal anesthesia (guinea pigs). Comparison of the effect of racemic and levo-nordefrin on the duration of anesthesia in parallel test.

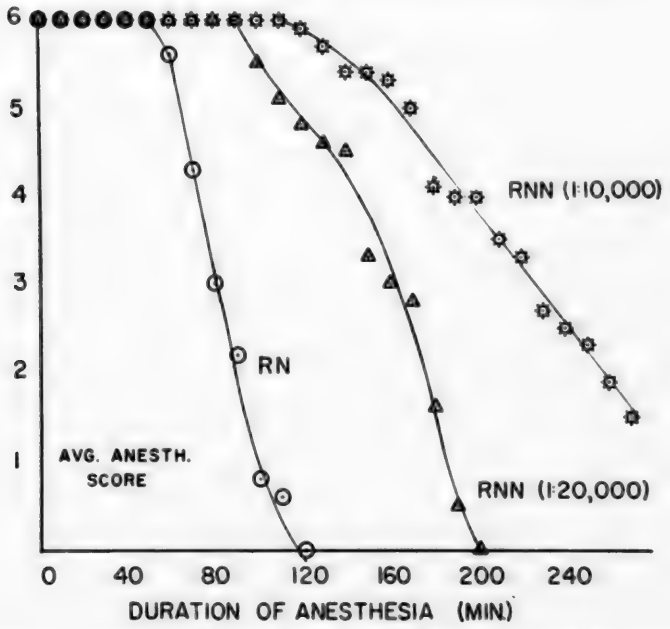

Fig. 4.—Intradermal anesthesia (guinea pigs). Effect of concentration of levo-nordefrin on duration of anesthesia.

The effect of levo-nordefrin in concentrations of 1:10,000 and 1:20,000 on the duration of anesthesia produced by a solution containing Ravocaine 0.4 per cent and Novocain 2.0 per cent is shown in Fig. 4. The tests were run in parallel on the same guinea pigs. The duration of the solution containing 1:20,000 levo-nordefrin was approximately double that of the same anesthetic solution without a vasoconstrictor. Doubling the concentration to 1:10,000 increased the duration an additional 30 per cent.

An anesthetic solution containing 1:10,000 of racemic nordefrin actually, of course, contains 1:20,000 of each optical isomer. On the basis of the findings reported here, removal of the 1:20,000 dextro-nordefrin should result in a modest reduction in toxicity and also a shortening of the duration of anesthesia.

SUMMARY

1. On dogs anesthetized with pentobarbital, levo-nordefrin was found 100 to 200 times more active than the dextro-isomer in raising the blood pressure. Epinephrine was 2 to 2.5 times more active than levo-nordefrin. In approximately equipressor doses, levo-nordefrin produced a somewhat greater increase in heart rate than epinephrine.

2. In equipressor doses, epinephrine was considerably more active than levo-nordefrin in stimulating the retractor penis whose motility was recorded simultaneously with the blood pressure. When equipressor doses of levo- and dextro-nordefrin were compared, dextro-nordefrin produced greater contractions of the retractor penis.

3. On the nonpregnant rat uterus in vitro, epinephrine was found 15 times more inhibitory than levo-nordefrin and the latter approximately 5,000 times more active than the dextro-isomer.

4. The intravenous $LD_{50} \pm$ S.E. of levo-nordefrin in white mice was 12.6 $\pm$ 2.7 mg./Kg. This compound was found 10 times more toxic than the dextro-isomer, and approximately one fifth as toxic as epinephrine.

5. Levo-nordefrin prolongs significantly the duration of local anesthesia. At a concentration of 1:20,000, duration was doubled when compared to the same solution without a vasoconstrictor. At a level of 1:10,000, the duration was increased an additional 30 per cent.

6. When levo-nordefrin 1:20,000 was compared with racemic nordefrin 1:10,000 in a solution of Ravocaine-Novocain, the duration was slightly longer with the racemic compound. This difference, however, is probably not of sufficient magnitude to be considered significant.

We wish to acknowledge the technical assistance of Mrs. Carol Priddle.

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FURTHER STUDIES ON THE NITROGEN CONTENT OF DENTAL PULP

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STUDY of the nitrogen content of dental pulp has been continued because of uncertainty about the uniformity of its distribution in this organ and similarity of concentration in different species of animals and in various stages of tooth development. Information on these points would be helpful in comparing quantitative data on respiratory activity of tissues, the reference standards of which were variously units of nitrogen or mass. In our laboratory it has been customary to express quantities of oxygen consumed or carbon dioxide produced in relation to dried tissue weight. However, there are occasions when the total nitrogen in a sample can be determined more readily than can the mass by direct methods. This is especially true of homogenates that have been prepared by the addition of cutting substances, such as ground glass, or of very small tissue samples where quantitative transfer to weighing receptacles may be incomplete. Under these or similar circumstances, if nitrogen values have previously been determined, it is a simple matter to analyze the sample for this element and calculate its original mass.

Ordinarily the respiratory activity of most tissues is sufficiently high so that unusual precautions in measuring gaseous exchange or mass are not required. This is not the case with dental pulp. Flieder and Fisher[1] reported the QO_2 of bovine pulp to be 0.56, S.D. 0.13. Slight errors in weight determinations on samples of this tissue can make a significant difference in the calculated oxygen quotient. In the instance of a bovine pulp, which often provides several samples, it is worth knowing if nitrogen distribution is uniform or not if the mass is to be calculated from total nitrogen.

Riedesel and Fisher[2] reported the nitrogen content of bovine and human dental pulp to be 0.1258 and 0.1050 mg. per milligram of dried tissue, respectively. These values were obtained without respect to the area of the organ from which the samples were taken. The samples of adult human dental pulp were often of pooled tissue from 2 or more teeth.

Preliminary studies on the regional distribution of pulpal nitrogen have been previously reported[3] but not published. An attempt was made to apply the ultramicro method of Tompkins and Kirk[4] to this problem. This method has a tested range of 0.5 to 20 μg of nitrogen and tissue samples of approximately

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100 μg were selected for analysis. After extensive trial the method was abandoned because it was discovered that unsatisfactory conditions for microweighing introduced a considerable error in the final results.

A second approach was made by nesslerizing the tissue and determining the nitrogen colorimetrically. Analyses of 139 samples taken from the pulpal horn, body, root, peripheral and central areas showed a fairly consistent mean content of total nitrogen.[3] However, the subsequent failure of some of us, who had not previously used this method, to duplicate the previous results, casts some doubts on the reliability of the method. Consultation with other workers who had had extensive experience with it disclosed that this experience was not unique. The use of nesslerization was, therefore, discontinued.

The present report is concerned with the nitrogen content of dental pulp as determined by a micro-Kjeldahl method. The concentration of this element in various regions of bovine and human pulp, its quantitative relation to the state of tooth development, and its concentration in the pulps of several species of animals has been investigated.

METHODS AND MATERIALS

The micro-Kjeldahl method used in this investigation required apparatus essentially as described by Jenden and Taylor.[5] It has a useful range of 5 to 250 μg of nitrogen. It was slightly modified by substitution of a rheostatically controlled electric heater in place of an open flame which resulted in improved distillation and more consistent results. This apparatus was more efficient than that originally employed[2] not only because of its smaller internal volume but because there were fewer joints through which ammonia might accidentally be lost. Ammonia was collected in 0.003 N sulfuric acid. Titration was done with 0.003 N sodium hydroxide. The end point of pH 6.8 was determined both by methyl red indicator and a pH meter. A careful check on the accuracy of the method was made routinely by analyzing a known sample of ammonium sulfate after every 2 samples of tissue.

Dried tissue samples of appropriate size were selected by rough weighing on a chainomatic balance in micro-weighing tubes, and dried again for at least 24 hours at 105° C. The tubes and contents were then cooled in a desiccator and final weighing was accomplished on a keyboard-operated micro-balance.

Bovine, sheep, goat, and pig dental pulps were obtained from freshly slaughtered animals after the bodies were exsanguinated. Adult human pulps were obtained from molars and premolars extracted for clinical reasons. Human infant pulps were secured at autopsy. Pulps from dogs were removed from molars extracted from animals sacrificed for that purpose. Monkey pulp tissue was derived exclusively from canine teeth supplied by The Radiobiological Laboratory of the University of Texas and the United States Air Force.

Whenever possible the pulps were removed from the teeth immediately, freed of debris with the aid of stereoscopic microscope, washed, and dried to

constant weight for at least 24 hours at 105° C. When it was not possible to extract the pulps immediately, the teeth were fixed in 10 per cent formalin since previous work[2] has shown that this does not have a significant effect on nitrogen analysis.

RESULTS

Because somewhat different method and apparatus were involved in this study, it was advisable to redetermine the mean nitrogen content of bovine dental pulp. This was accomplished by analyses of 74 samples without regard to the anatomic regions from which they were obtained. Where individual sample sizes exceeded the limits of the method, aliquots of the digested samples were employed. Following this general analysis, total nitrogen determinations were done on 12 samples of bovine pulp horns, 12 samples of pulp body, and 17 samples of the radicular pulps. The results are presented in Table I.

TABLE I

BOVINE PULP NITROGEN CONTENT DETERMINED BY MICRO-KJELDAHL METHOD

AREA	NO. OF SAMPLES	DRIED SAMPLE SIZE (MG.)	MEAN (MG. N/MG. DRY WT.)	S.D.	RANGE (MG. N/MG. DRY WT.)
General	74	0.66-31.07	0.118	0.007	0.102-0.132
Horn	12	0.86-1.25	0.117	0.008	0.102-0.130
Body	12	0.85-1.21	0.115	0.005	0.108-0.121
Root	17	0.66-1.65	0.118	0.007	0.109-0.131

Inasmuch as the data derived from the preceding analyses did not precisely duplicate the nitrogen values originally reported[2] the studies were extended to human adult and infant pulp tissue. These results are shown in Table II. The much smaller size of adult human dental pulp made it impossible to divide the organ as was done for bovine pulp if pooling was to be avoided. The pulps of the teeth of newborn infants do not present the clearly defined anatomic regions apparent in mature teeth.

TABLE II

HUMAN PULP NITROGEN CONTENT DETERMINED BY MICRO-KJELDAHL METHOD

AREA	NO. OF SAMPLES	DRIED SAMPLE SIZE (MG.)	MEAN (MG. N/MG. DRY WT.)	S.D.	RANGE (MG. N/MG. DRY WT.)
Tissue From Adult Premolar and Molar Teeth					
Crown	23	0.43-0.96	0.122	0.011	0.092-0.140
Root	20	0.46-1.26	0.130	0.016	0.099-0.149
Tissue From Infant Tooth Germs					
General	14	1.06-1.46	0.117	0.006	0.107-0.128

The slight but suggestive difference between the mean total nitrogen of adult and infant pulps was examined more fully in more easily obtainable bovine teeth. Those molars with fully formed roots with constricted apical

foramina were designated as "old," those with root formation in progress as "young," and those with no evidence of root formation as "embryonic." The results of nitrogen determinations on these specimens are indicated in Table III.

TABLE III

BOVINE PULP NITROGEN CONTENT DETERMINED BY MICRO-KJELDAHL METHOD

DEVELOPMENT STATE	NO. OF SAMPLES	DRIED SAMPLE SIZE (MG.)	MEAN (MG. N/MG. DRY WT.)	S.D.	RANGE (MG. N/MG. DRY WT.)
Embryonic	28	0.73-1.19	0.109	0.008	0.094-0.118
Young	23	0.74-1.25	0.114	0.004	0.106-0.122
Old	27	0.72-1.15	0.118	0.008	0.100-0.134

They would appear to confirm the trend toward higher nitrogen content in more mature dental pulps that was observed in the human material. The increase is not great, however.

In extending these studies to the pulp tissue of other species of mammals no further attempt was made to investigate the relation of maturation and anatomic site to nitrogen content. Availability of the animals and the size of individual pulps determined what species were included in this series. The results of this phase of the study are shown in Table IV. A remarkable similarity is apparent in comparing the different tissues.

TABLE IV

NITROGEN CONTENT OF SEVERAL SPECIES OF MAMMALS DETERMINED BY MICRO-KJELDAHL METHOD

SPECIES	NO. OF SAMPLES	DRIED SAMPLE SIZE (MG.)	MEAN (MG. N/MG. DRY WT.)	S.D.	RANGE (MG. N/MG. DRY WT.)
Monkey	38	0.73-1.38	0.118	0.008	0.098-0.129
Pig	18	0.98-2.01	0.113	0.007	0.098-0.124
Sheep	16	0.93-1.37	0.119	0.004	0.112-0.124
Dog	20	0.70-1.62	0.115	0.006	0.103-0.126
Goat	20	0.81-1.32	0.108	0.008	0.096-0.131

DISCUSSION

Graphic presentation of the foregoing data (Fig. 1) illustrates most clearly the very slight variation of mean total pulpal nitrogen arising from differences in species, state of tooth development, and anatomic location. The mean values of all series of determinations, plus or minus the standard deviation, all overlap at 0.115 mg. of nitrogen per milligram of dried tissue. The mean value of the means of all series is 0.117 mg.

The unusually high value and extreme range shown by adult human pulp possibly may be more apparent than real. This tissue was often observed to contain greater quantities of blood than was true for the other materials. The retained blood was usually concentrated in the radicular pulp. It is suspected that this circumstance is somehow related to vascular modifications incident to the process of extraction. It did not occur where death or exsanguination

preceded extraction as in the autopsy material or in material obtained from the slaughtering plant. The lower extreme of the range of adult human pulp nitrogen could have been the result of undetected dystrophic calcifications that increase the dry weight without contributing proportionately to the nitrogen content.

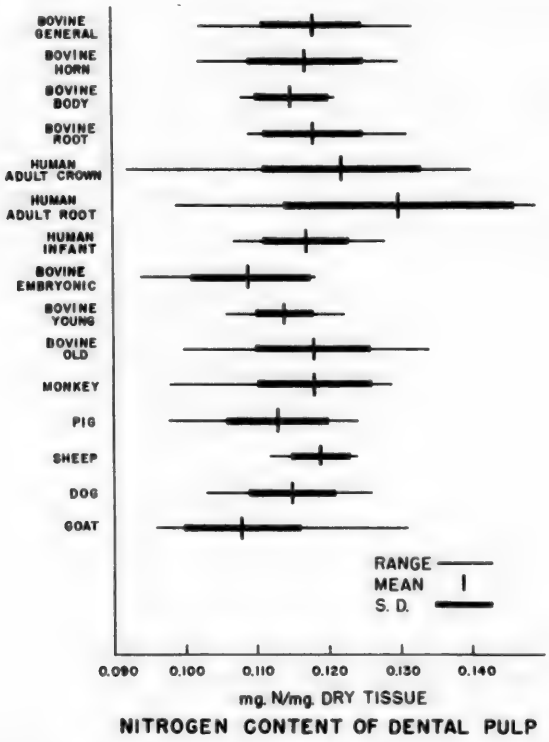

Fig. 1.

The apparent slight increase in pulpal nitrogen that seems to occur as this tissue becomes more mature may account for the closer correspondence of most values to that of human infant pulp than to adult pulp. Chronologically and physiologically the larger proportion of adult human teeth were older than the others.

The suggestion of a species peculiarity in the nitrogen content of dental pulp that was noted in earlier studies[2] has not been substantiated in the present investigation. The difference in the values presented in this and the previous report is believed to be attributable to refinements in the analytical method. Application of the accomplishments of this work to the purposes for which it was undertaken provides a practical and satisfactory result if the mean nitrogen content of dental pulp is considered to be 0.117 mg. per milligram of dried tissue.

SUMMARY

Analyses of total nitrogen in human, bovine, monkey, sheep, pig, goat, and dog dental pulp show no significant species differences. The nitrogen content appears to be fairly uniform in the various major anatomic regions of this organ but the total nitrogen seems to increase very slightly as the tissue becomes more mature. The mean total pulpal nitrogen determined by this study is 0.117 mg. per milligram of dried tissue.

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EARLY PULP CHANGES IN THE TEETH OF A DOG FOLLOWING FULL CROWN PREPARATIONS

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INEVITABLY associated with crown preparation of a tooth are increases of pressure, temperature, and vibration. The effects of pressure on the dental pulp have not been comprehensively investigated. As an incidental finding, we have noted that human teeth which have been extracted with forceps invariably presented an area of odontoblastic destruction. This area coincided precisely with the dentinal tubules which had been exposed and crushed by the beaks of the forceps. In some cases, there was, in addition, some capillary hemorrhage between the odontoblasts. It appeared that for each tubule exposed to pressure, the corresponding odontoblast was ruptured. Others have noted and recorded these same phenomena.[1, 2, 3] The effects of pressure and heat on the dental pulp cannot be separated since there apparently exists a direct correlation between the 2 factors. The greater the pressure of the cutting instrument, the higher the frictional heat developed.[3–6] Investigators[6–12] have measured the rise in temperature within a tooth when it has been cut with stones, burs, and diamond instruments. The implications that these increases are capable of producing serious irreparable damage to the dental pulp were not borne out by the investigations of Lisanti and Zander.[13] They found that temperatures of 125° F. to 600° F. applied to the dentin of dogs' teeth for 5 seconds to 1 minute were capable of producing changes in the pulp, ranging from separation of the odontoblastic layer from the dentin to destruction of the odontoblastic layer and blister formation within the pulp. In all cases, however, healing took place as time elapsed and within 2 months there was repair with no pulp deaths. In full crown preparation, however, where all the dentinal tubules are exposed, and the total drilling time exceeds 1 minute, repair might not be so complete. When extra increments of irritation in the form of heat and pressure from modelling compound are added, the pulp reaction might not be so favorable. Lisanti and Zander[13] listed many factors which may influence the amount of heat produced during operative procedures. An important method of controlling the heat produced is by the use of water coolants on the teeth while cutting is taking place.[5, 6, 9]

The purposes of this investigation were (1) to study the effects of crown preparation with rotary tools run at comparatively low speeds on the pulps of

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the teeth of a dog, (2) to discover if there were differences in pulp reaction with and without water cooling of the teeth at the time of their preparation, and (3) to determine the effects of several different kinds of pressure on the dental pulp, both with and without the heat ordinarily applied to the dentin for impression taking.

EXPERIMENTAL PROCEDURE

A healthy dog, exact age unknown, was anesthetized by intravenous administration of Pentothal sodium. Crown preparations, simulating those made on human teeth for full cast crowns, were then made on 10 teeth. The left side was prepared first, beginning with the upper left carnassial. Following this, the upper left canine, lower left second molar, lower left carnassial, and lower left canine were prepared in that order. Next, the right side was prepared, beginning with the upper right carnassial. Then prepared in sequence were the upper right canine, lower right second molar, lower right carnassial, and the lower right canine. The entire operative procedure took about 2 hours. The time, elapsing between tooth preparation and death of the animal, varied for each tooth. The elapsed time between death and preparation for the last tooth to be prepared (lower right canine) was approximately ½ hour, for the first tooth to be prepared (upper left carnassial) about 2½ hours.

In these crown preparations, most of the enamel and some of the dentin was ground from the teeth with diamond discs and wheels mounted in a handpiece operated by a portable dental engine. Very little pressure was used, although the exact amount was not measured. Approximately the same amount of pressure was applied to each tooth. The speed of the engine was approximately 3,500 r. p. m. All the teeth on the left side were ground under a stream of tepid water, approximately 37° C. The teeth on the right side were ground with the same instruments, but without the stream of water.

On 2 teeth (lower left carnassial and lower right carnassial), nothing further was done. The following additional procedures were employed in the remaining 8 teeth. On 2 teeth (upper left canine and upper right canine), impressions were taken, using light pressure, by means of copper bands filled with Plasticine. Light pressure means that the lightest amount of pressure necessary to obtain an impression of the tooth was used. Measurements of the exact amount of pressure employed in this or any of the subsequent procedures were not made. Two teeth (upper left and upper right carnassials) had impressions taken with copper bands filled with Plasticine, employing the maximum amount of digital pressure possible. The maximum amount of pressure means that the thumb was pressed over the impression band and on the tooth with all the muscular force possible. In 2 teeth (lower left and lower right second molars), impressions were taken with copper bands filled with modelling compound, heated to a temperature of 40° to 45° C., employing light pressure. In the remaining 2 teeth (lower left and lower right canines), impressions were taken with hot modelling compound (40° to 45° C.), using the maximum amount of digital pressure possible. Table I summarizes the procedures employed.

TABLE I
PROCEDURES EMPLOYED ON DOG'S TEETH

WITH WATER	WITHOUT WATER	PROCEDURE
Upper left first molar	Upper right first molar	Controls; no treatment
Lower left carnassial	Lower right carnassial	Crown preparation only; no impression
Upper left canine	Upper right canine	Crown preparation; impression with Plasticine (room temp.), light pressure
Upper left carnassial	Upper right carnassial	Crown preparation; impression with Plasticine (room temp.), maximal pressure
Lower left second molar	Lower right second molar	Crown preparation; impression with modelling compound (40°-45° C.), light pressure
Lower left canine	Lower right canine	Crown preparation; impression with modelling compound (40°-45° C.), maximal pressure

The pulp of the upper left canine was accidentally exposed during the preparation. The animal was sacrificed immediately afterward by administration of an overdose of anesthetic intravenously. The mandible and maxillae were removed with a band saw. The teeth were separated with the saw and about one third of the root ends were cut off, thereby exposing the pulp canals to the fixative. The teeth were then placed individually in bottles containing 10 per cent formalin. Following decalcification, frozen sections were prepared in a horizontal plane. These sections were then stained with hematoxylin and eosin and mounted on slides for microscopic examination.

FINDINGS

Untreated Controls.—

The odontoblastic layer of the pulp of the upper left first molar was uniform in width. The odontoblasts were regularly arranged in palisade formation, parallel with one another and in continuous contact (Fig. 1). The capillaries between the odontoblasts were not dilated. There was no hemorrhage. Within the connective tissue of the pulp, one relatively fibrosed area was discovered in which there was an increase in collagen fibers as compared to the rest of the pulp. There was also some slight hemorrhage in that area.

The upper right first molar exhibited a pulp with an odontoblastic layer of uniform width in which the cells were regularly arranged. Within the body of the pulp, there were several small areas of hemorrhage. There was a slight amount of blood vessel dilation throughout the entire pulp. The connective tissue cells of the pulp were relatively normal.

Teeth Subjected to Crown Preparation Only.—

The lower left carnassial was prepared under a stream of water. The capillaries in the odontoblastic layer were dilated and congested. In many areas, the blood vessel walls were ruptured and the odontoblasts were destroyed by the resultant hemorrhage. In a few areas the hemorrhage was of massive proportions, extending into the body of the pulp. Deeper within the pulp, the blood vessels were hyperemic and the tissue was edematous. Numerous macrophages were found near the areas of edema. Some calcific concretions were present within the body of the pulp.

The lower right carnassial was prepared without water cooling. Hemorrhages within the odontoblastic layer were profuse, multiple, and extensive throughout. The odontoblasts were destroyed in many areas as a result. In other areas, the odontoblastic layer appeared compressed, thinner, and shrunken. Hemorrhages were also observed throughout the body of the pulp. Many macrophages were found among the cells of the pulp.

Teeth Subjected to Crown Preparation and Impression With Plasticine Using Light Pressure.—

The upper left canine, which was prepared under water, showed a marked hyperemia of the blood vessels of the pulp. In addition, the capillaries, both between the odontoblasts and within the body of the pulp, were ruptured in several areas with resultant hemorrhage (Fig. 2). The odontoblastic layer was shrunken and narrowed in several areas. The odontoblastic nuclei were pyknotic in these regions and the palisade arrangement of the odontoblasts was altered. Most of the pulp cells were rounder and appeared to be undergoing degenerative changes. Intercellularly, there was eosin-staining exudate.

In the pulp of the upper right canine, which was not cooled by water during preparation, there was an abundance of pinkish-stained exudate indicating that there was a considerable degree of capillary permeability. The blood vessels were hyperemic. The odontoblastic layer was reduced in width in one region. Here the odontoblasts were arranged in disorderly fashion. The tissue in this region appeared edematous. In most regions, vacuoles had replaced the pulpo-dental membrane (Fig. 3).

Teeth Subjected to Crown Preparation and Impression With Plasticine Using Maximal Pressure.—

The pulp of the upper left carnassial, which was prepared under water, showed a moderate hyperemia. There were fairly extensive areas of hemorrhage around the capillaries of the odontoblastic layer with resultant destruction of odontoblasts. In all other regions, the capillaries between the odontoblasts were dilated and congested. Many vacuoles were found between odontoblasts and in some regions the odontoblastic nuclei were lying on the predentin, as if they were about to be sucked into the dentin. In one area, the odontoblasts had lost their orderly arrangement and the nuclei were pyknotic.

The upper right carnassial, prepared without water, showed hyperemic blood vessels within the pulp. Capillary hemorrhages within the odontoblastic layer and under it were prevalent. The odontoblastic layer was narrowed in one region and the palisade arrangement of the cells was changed. Here the cells tended to become more vacuolated and nuclei were pyknotic. In one region, odontoblastic nuclei were aspirated into the dentin.

Teeth Subjected to Crown Preparation, Impression With Modelling Compound (40°-45° C.) With Light Pressure.—

The pulp of the lower left second molar, in which the crown had been prepared under water, showed a marked amount of capillary hemorrhage within the odontoblastic layer and also within the body of the pulp. The hemorrhage was so extensive in certain areas that it extended into the dentin.

Fig. 1.—Photomicrograph of pulpo-dentinal region of untreated control. Palisade arrangement of odontoblasts within odontoblastic layer (*OdL*). Odontoblasts have oval elongated nuclei (*N*) containing chromatin granules. Capillaries between odontoblasts and within body of pulp (*P*) are not dilated or congested. (Orig. mag. ×600.)

Fig. 2.—Photomicrograph of pulp (*P*) of upper left canine. Crown preparation made with water cooling. Hemorrhages of capillaries between odontoblasts and also large hemorrhage within body of pulp (*H*). Plasma within dilated blood vessels (*pl*). (Orig. mag. ×180.)

Fig. 3.—Photomicrograph of pulpo-dentinal region of upper right canine. Higher magnification of odontoblastic layer of pulp (*P*). Loss of palisade arrangement of odontoblasts (*OdL*). Pyknosis of odontoblastic nuclei (*pk*). Vacuoles destroying pulpo-dental membrane. Dilatation of capillaries between and below odontoblasts (*dc*). (Orig. mag. ×600.)

Fig. 4.—Photomicrograph of pulp of lower left second molar, prepared under a stream of water. Higher magnification of pulpo-dentinal region. Loss of palisade arrangement of odontoblasts (*OdL*). Red blood cells (*rbc*). Loss of pulpo-dentinal membrane. Aspiration of odontoblastic nuclei (*Od*) into the dentin (*D*). (Orig. mag. ×600.)

Fig. 5.—Photomicrograph of pulp of lower right second molar, prepared without water. Complete destruction of odontoblasts and connective tissue cells of pulp by hemorrhage (*H*). (Orig. mag. ×180.)

Fig. 6.—Photomicrograph of pulp of lower left canine. This tooth was prepared under water. Loss of palisading of odontoblasts. Narrowing of odontoblastic layer (*OdL*). Pyknotic nuclei (*pk*). Increase of number of capillaries. Flattened cells due to edema (*E*). (Orig. mag. ×600.)

There was accompanying hyperemia of the blood vessels within the pulp. The capillaries were choked with polymorphonuclear leukocytes. This was especially evident within the capillary loops between the odontoblasts. In some areas the odontoblastic nuclei were seen to have been aspirated in the dentin (Fig. 4).

The lower right second molar was prepared without water cooling. The pulp of this tooth showed extensive, massive hemorrhages throughout. Quite a large amount of pulp tissue was destroyed as a result (Fig. 5). The odontoblasts also were destroyed extensively by hemorrhage. In other regions the nuclei of the odontoblasts were pyknotic. Large numbers of polymorphonuclear leukocytes were present in the blood vessels. In some vessels, margination of the leukocytes was evident. Edema was present in some areas. Odontoblastic nuclei were found aspirated into the dentin in various regions.

Teeth Subjected to Crown Preparation, Impression With Modelling Compound (40°-45°) With Maximal Pressure.—

The pulp of the lower left canine was markedly hyperemic. Many blood vessels were distended with plasma. The edema was striking. Blood vessels contained polymorphonuclear leukocytes. Pulp cells in some areas were flattened due to the edema (Fig. 6). The odontoblastic layer was destroyed in one area. The cells had lost their palisaded appearance and were stratified linearly. Their nuclei were pyknotic. Here the width of the layer was reduced. In certain regions, nuclei rested against the dentin, as if about to be aspirated.

The lower right canine was prepared without water cooling. The pulp was hyperemic. Many polymorphonuclear leukocytes were present within the blood vessels and these were pavemented against the walls of the vessels. There was a large quantity of plasma within the vessels and extracellularly. Polymorphonuclear leukocytes were especially prominent in the vessels of the odontoblastic layer. The odontoblasts were compressed on one region and their nuclei were pyknotic. In some regions nuclei were against the predentin as if they were about to be aspirated. There were several small areas of hemorrhage just under the odontoblastic layer. Most of the pulp cells were rounded, as if they had been compressed.

DISCUSSION

The odontoblast is apparently quite sensitive to irritation. Preparation of a tooth for a crown resulted in some change in the odontoblastic layer in every instance. Although not measured, most preparations were relatively shallow, yet the odontoblasts were either reduced in number (reduced width of odontoblast layer), or altered morphologically (pyknosis), or disarranged (loss of palisading). A striking and prominent feature of most preparations was the hemorrhage within the pulp and especially of those blood vessels within the odontoblastic layer. This was profuse enough to cause degeneration of the odontoblasts and connective tissue cells of the pulp in multiple areas.

Pressure applied to the dentin by means of impression material produced some significant changes. The odontoblasts were injured more severely when

pressure was applied to the dentin. In 3 of the preparations subjected to pressure, odontoblastic nuclei were seen to be aspirated into the dentin. In 2 other preparations which were subjected to pressure, there was a suggestion that aspiration of nuclei was about to occur. This indicated that pressure was apparently instrumental in producing aspiration of odontoblasts into the dentin.

When heat was applied to the dentin in addition to the pressure, other changes became evident. There was profuse diffusion of plasma through the blood vessel walls with resultant edema of the pulp. Polymorphonuclear leukocytes began to appear within the blood vessels in increased numbers and there was margination of these leukocytes along the vessel walls. Neutrophilic leukocytes also became evident in the capillaries between the odontoblasts. The odontoblastic nuclei became pyknotic, indicative of imminent degeneration of the cells.

Unfortunately, due to the variation in time, one could not compare teeth which were prepared under water with those prepared without the use of water cooling. There were no significant differences between the pulp reactions of the teeth of the 2 groups. The reason for this might be because of the difference in time between preparation of the teeth and death. The teeth which were prepared with the use of water cooling were operated upon first, hence the pulps had a longer time for reaction before the tissues were fixed. Those prepared without water were prepared last and were all placed in fixative within about 1¼ hours to ½ hour after preparation. Thus, it is likely that time did not permit more severe reactions to take place.

The results of this experiment essentially agree with those of James, Schour, and Spence[14] who noted that, 3 hours after cavity preparation and filling with gutta-percha, the odontoblasts were injured, hemorrhages were present in the deep pulpal tissue, and neutrophils and macrophages began to appear, among other changes.

The damage produced by full crown preparation is proportionately greater than that produced by cavity preparation because of the greater number of dentinal tubules exposed.

SUMMARY AND CONCLUSIONS

Ten teeth of 1 dog were subjected to full crown preparations, similar to those made for human teeth, by means of rotary tools revolving at approximately 3,500 r. p. m. For 2 teeth nothing further was done. Impressions were taken of 2 teeth with copper bands containing Plasticine, employing light pressure. In 2 teeth, Plasticine impressions were taken employing maximal pressure. Using light pressure, 2 teeth were subjected to pressure with modelling compound, heated to 40° to 45° C. The same thing was done with the remaining 2 teeth employing maximal pressure. One tooth of each pair was prepared under a stream of tepid water. The other tooth was prepared without water cooling. The animal was sacrificed 2½ hours after the beginning of the experiment. Studies of horizontal sections of the teeth revealed that crown preparations were

responsible for the significant changes. When additional pressure was applied to the dentin by means of Plasticine, the odontoblasts were sucked or pumped into the dentin in some areas.

The addition of heat, by means of the modelling compound impression, caused an increase in capillary permeability, as indicated by the presence of large quantities of plasma within the pulp tissue. Also evident was a larger number of neutrophilic leukocytes which were marginated along the blood vessel walls. These leukocytes were especially noticeable in the capillaries between the odontoblasts.

The differences of pulp reaction between those teeth prepared under water and those prepared without water cooling were not distinct. It is believed that the variability of time between preparation of the teeth and death could account for this.

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EXPERIMENTAL PULPOTOMIES IN RAT MOLARS

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THE purpose of this investigation was (1) to develop further a technic for performing pulpotomies on rat molars, (2) to study the pattern of pulpal healing following amputation, and (3) to compare the effects of calcium hydroxide and of zinc oxide and eugenol on the pattern of pulpal healing in the molars of rats.

It has long been realized that pulpal amputation must be carried out and studied on a large scale under precisely controlled laboratory conditions in order to evaluate more accurately the process of pulpal healing. For this purpose, the rat is a suitable animal because it is easily available in large numbers and is comparatively cheap. However, because of the small size of the rat's teeth, it first becomes necessary to evolve a reliable method of performing pulp amputation and pulp capping in the rat molar. Furthermore, this technic should be easily reproduced by other investigators in this field.

Mohammed and Schour,[1] Maurice and Schour,[2] Perreault, Massler, and Schour,[3] and Silberkweit, Massler, Schour, and Weinmann[4] have evolved simple methods for operating on the incisor and molar teeth of rats in order to study the pulpal reaction to various filling materials. Massler, Perreault, and Schour[5] and O'Malley[6] adapted the above methods to the study of the effects of pulpal amputations in the continuously growing incisor of the rat.

Miyamoto[7] successfully performed surgical amputations on the pulps of rat molars. His investigation has made it possible to develop further a technic for performing pulpotomies and to study many of the features of pulpal healing in a molariform tooth of limited growth.

METHODS AND MATERIALS

Pulp amputations were performed in 122 upper first molars of 61 male hooded rats. Forty-five animals were 60 to 90 days of age, while 9 were 30 days old, and 7 were 220 or 360 days of age. The 2 latter groups were part of a pilot study on the effects of age on the rate of pulpal healing.

The animal was anesthetized by an intraperitoneal injection of Pentothal sodium, 1 per cent, and placed on an operating table on its back. The tongue and cheeks were reflected from the field of operation. Instruments and burs were modified to suit the size of the tooth as described by Maurice and Schour.[2] Aseptic conditions were attempted but not always achieved.

An attempt was made to remove dentin splinters with an air spray, but the histologic sections showed this to have been unsuccessful in a number of cases. If there was marked hemorrhage, a paper point was used to remove

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the excess whole blood, but no deliberate attempt was made at the time of the operation to influence the bleeding time or the character of the clot. Some exposures were nonbleeding or had very little bleeding. The presence of a blood clot and its character were finally determined histologically.

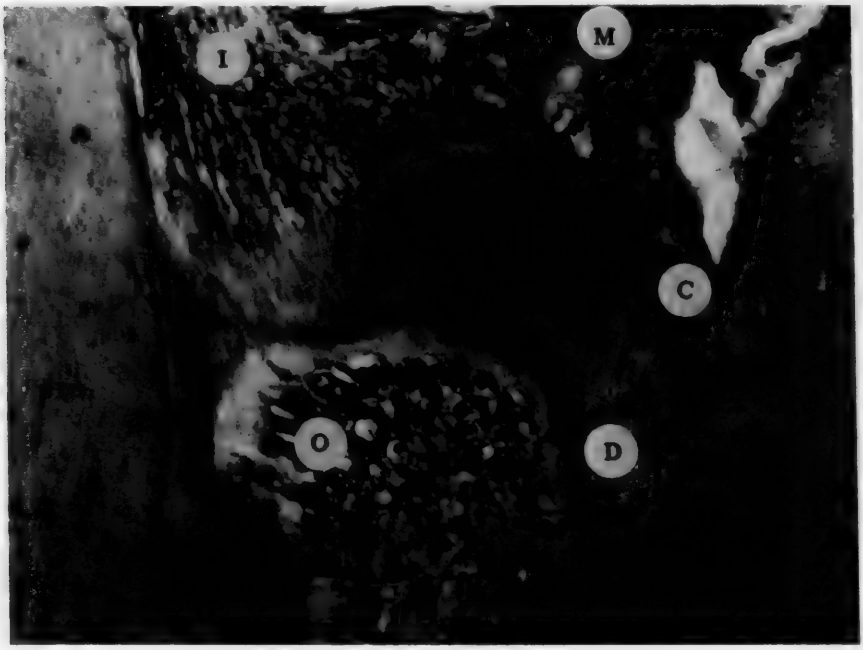

Fig. 1.—Photomicrograph showing pulpal healing 28 days after surgical amputation and covering with calcium hydroxide (Hematoxylin and eosin; Orig. mag. ×300). Note characteristic zones. *M*, medicament; *I*, zone of pulpal degeneration; *C*, primary calcific bridge; *D*, permanent dentin bridge; *O*, odontoblastic layer.

Two main operative approaches were investigated, one from the mesial surface of the first molar and the other from the occlusal surface. It was found that the occlusal approach via the middle pulp horn was superior to the mesial approach for producing an exposure which could be controlled in terms of size and depth. Only half of the 28 fillings placed in the mesial surface were retained while 89 per cent of the 94 fillings placed in the occlusal surface were retained, even after 28 days.

The medicaments used were calcium hydroxide and zinc oxide and eugenol. Each medicament was placed in 61 teeth, alternating on right and left side in successive animals. Each was placed in direct contact with the pulp tissue wherever possible. When calcium hydroxide was used, the powder was dusted into the cavity, covered with paraffin wax and then sealed with amalgam. The zinc oxide and eugenol cappings were also sealed with amalgam.

Approximately equal numbers of animals were sacrificed at intervals of 7, 14, 21, and 28 days following the operation. The animals were decapitated and the jaws fixed in 10 per cent formalin. The specimens were decalcified,

embedded in paraffin or celloidin, and sectioned at 10 microns. They were stained with hematoxylin and eosin or with Mallory's triple connective tissue stain.

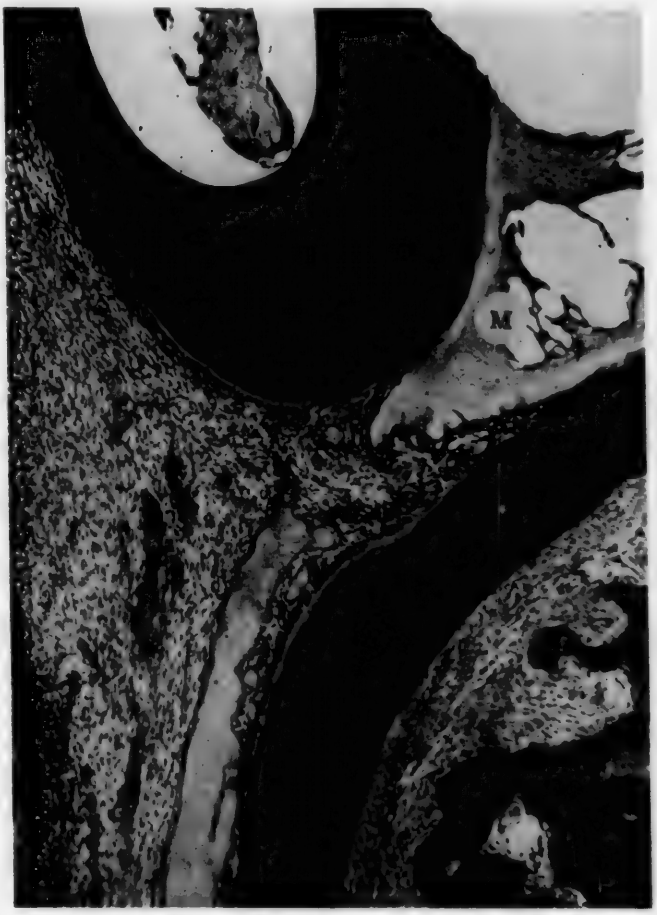

Fig. 2.—Photomicrograph showing pulpal reaction to zinc oxide and eugenol 7 days after surgical amputation (Hematoxylin and eosin; Orig. mag. ×80). Note zinc oxide and eugenol (*M*) in direct contact with pulp tissue and increased vascularization.

FINDINGS

Pattern and Chronology of Healing.—

The most important finding was the fact that healing followed a definite pattern under large or small exposures and under both types of medicament used. Healing took place by the formation of different reaction zones below the amputation site. These were seen clearly in specimens obtained at 21 to 28 days. Fig. 1 shows the characteristic zones: the layer of medicament, the zone of pulpal injury and degeneration, the primary calcific bridge, the permanent bridge of reparative dentin, the odontoblastic layer, and normal pulp tissue.

Medicament layer: The zone above the area of amputation was usually composed of remnants of the medicament either alone or mixed with blood or debris. Zinc oxide and eugenol tended to remain above the layer of whole blood and did not mix with it, while calcium hydroxide was usually mixed with blood and caused its hemolysis (Figs. 2, 3, and 6).

Fig. 3.—Photomicrograph showing pulpal reaction to calcium hydroxide 7 days after surgical amputation (Hematoxylin and eosin; Orig. mag. ×80). Note that medicament (*M*) is admixed with hemolyzed blood. I_1 indicated coagulated pulp tissue. Zone of degeneration (I_2) is prominent and there is seen beginning of formation of primary calcific bridge at *C*.

When a layer of blood or tissue exudate was found between the medicament and the pulp tissue, this tended to modify and dilute the action of the medicament.

Zone of pulpal injury and degeneration: The superficial portion of the amputated pulp was usually macerated, necrotic or, coagulated. The depth and degree of injury in this area depended on the amount of trauma caused by the operation and the coagulating effect of the medicament applied. A

thick layer of coagulated pulp tissue developed under calcium hydroxide, while under zinc oxide and eugenol little or no necrosis of the superficial layer of amputated pulp tissue was apparent (Figs. 2 and 3).

The deeper portion of this zone was characterized by degenerative changes. The intercellular matrix (pink-staining) appeared to be more prominent and enclosed degenerating cells (Fig. 4). This layer was first seen at 7 days and became more prominent on longer survival.

Primary calcific bridge: At approximately 14 days, below the zone of injury and degeneration, there was seen a blue-staining layer of dystrophic calcification. It was characterized by the appearance of large degenerating pulpal cells entrapped within a dense network of fibrous strands (Figs. 1, 4,

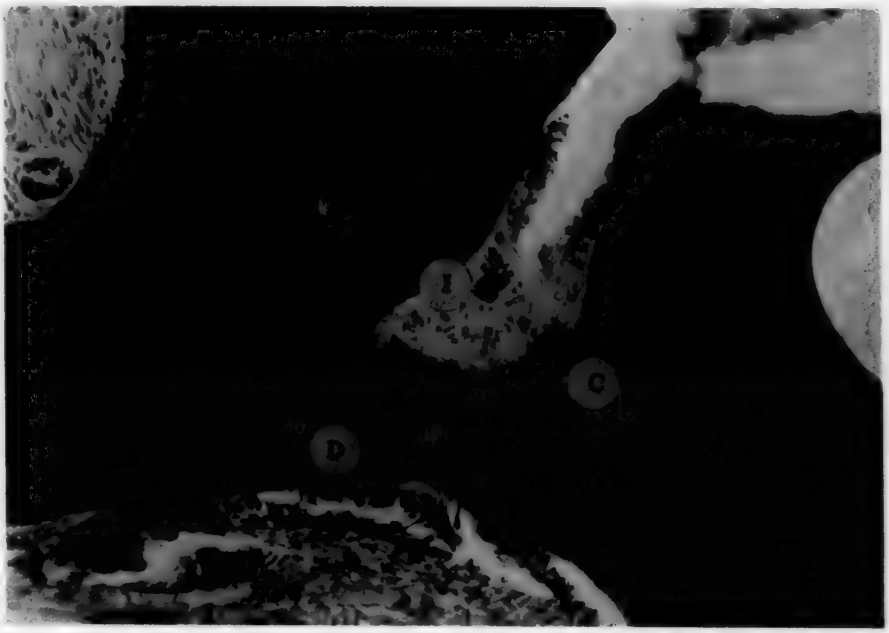

Fig. 4.—Photomicrograph showing pulpal reaction to zinc oxide and eugenol 21 days after surgical amputation (Hematoxylin and eosin; Orig mag. ×130). Note the characteristic zoning. *I*, zone of degeneration; *C*, primary calcific bridge. Permanent dentin bridge (*D*) shows well-organized, tubular arrangement.

and 5). These fibers (or their enveloping cementing substance) stained a deep blue color with hematoxylin, leading to the conclusion that they were undergoing calcification. The term dystrophic refers to the fact that the er trapped cells were in a state of degeneration.

The zone of dystrophic calcification always appeared between the zone of pulpal degeneration above and the normal pulp tissue below. It thus formed a protective calcific bridge over the vital pulp tissue and preceded the appearance of the true dentin bridge formed later by odontoblastic cells. For this reason it is referred to as the "primary calcific bridge." In thickness, it ranged from a thin line (Fig. 1) to a thick, well-differentiated zone (Fig. 4).

Its size appeared to depend on the nature of the zone above. When the zone of pulpal injury above showed a slow progressive and mild degeneration, the primary calcific bridge formed slowly and late, was not well organized and tended to be wide. When the zone above showed a rapid, coagulating necrosis, the primary calcific bridge appeared early, was dense, well organized and narrow. In the latter instance, formation of the permanent dentin bridge began early, soon after the appearance of the primary calcific bridge.

Fig. 5.—Photomicrograph showing wide primary calcific bridge under calcium hydroxide 28 days after surgical amputation (Hematoxylin and eosin; Orig. mag. ×300). Note large degenerating cells entrapped within calcified fibrous strands. *I*, zone of degeneration.

The presence of a primary calcific bridge was the most constant and characteristic feature at 14 days after pulpal amputation. It appeared to act as an organizer for the cells involved in the dentin formation and bridging that followed. It obviously represented an early walling off of the injured and degenerating portion of the pulp from the vital pulp tissue below.

Permanent bridge of reparative dentin: At 21 days, it was possible to determine whether complete pulpal healing would or would not take place. Those specimens, in which a thin primary bridge had formed, usually showed

a well-developed layer of true dentin bridging the site of amputation. Specimens which showed a wide, irregular primary calcific bridge were much slower in stimulating the formation of a dentin bridge. In such cases, the amount of secondary dentin formed at 21 days was much smaller (compare Figs. 4 and 5).

That portion of the permanent dentin bridge which was formed first, in contact with the zone of dystrophic calcification, tended to be irregular in structure (Fig. 4). The portions formed later were more regular.

The dentin bridge extending across the wound site was made up of 2 components. The central portion was formed by newly differentiated cells and therefore tended to be irregular in structure. The lateral portions were formed by old odontoblasts lining the wall of the dentin adjacent to the site of amputation. These cells seemed to be stimulated to renewed activity by the amputation and formed a thick layer of regular, tubular dentin which, under a small exposure, almost bridged the entire gap (Figs. 1 and 4).

Vital Pulp Reaction.—

Vascular reaction: A very prominent feature of the healing pattern was the increased vascularization of the pulp tissue immediately below the zones of reaction. It was very prominent at 7 days and less prominent, but still present, at 14, 21, and even at 28 days.

At 7 days after amputation, there was a marked increase in the number of capillaries in the pulp just below the zone of injury (Figs. 2 and 3). These vessels were always dilated and at times ruptured causing small hemorrhages into the pulp tissue.

The blood vessels seemed to retreat as the primary calcific bridge and the permanent dentin bridge were formed and increased in thickness. Finally, these vessels formed a subodontoblastic plexus below the cells that formed the permanent bridge. The increased peripheral vascularization may be a reaction of the vital pulp to the trauma of the operation but, since it is present even in later specimens, it is more likely to be an integral part of the healing process.

Cellular reaction: The odontoblasts at the site of amputation were severely injured and consequently degenerated. However, at a slight distance from the site of amputation, the odontoblasts were strongly stimulated and produced large amounts of secondary dentin rapidly, forming the lateral margins of the dentin bridge (Figs. 2, 3, and 4). At a farther distance, the odontoblasts appeared unaffected by the operation.

The primary calcific bridge was probably the product of degenerating cells whereas the permanent dentin bridge was formed as a result of vital cellular processes. It appeared that approximately 14 days were required for new odontoblasts to differentiate and at least 21 to 28 days before they produced a regular type of tubular dentin which formed the main portion of the permanent dentin bridge.

Comparison Between Calcium Hydroxide and Zinc Oxide and Eugenol.—

The basic pattern of pulpal healing described above was observed under both calcium hydroxide and under zinc oxide and eugenol. Minor differences

were evident at 7 and 14 days. Calcium hydroxide had a distinct necrotizing or coagulating effect on the superficial portion of the amputated pulp and on any intervening blood or exudate. This appeared to cause an early appearance (often at 7 days) of the zone of dystrophic calcification (Fig. 3).

When the zinc oxide and eugenol were in direct contact with the pulp, the underlying tissue appeared to be normal or it contained a large number of polymorphonuclear leukocytes (Fig. 6). When polymorphonuclear leukocytes

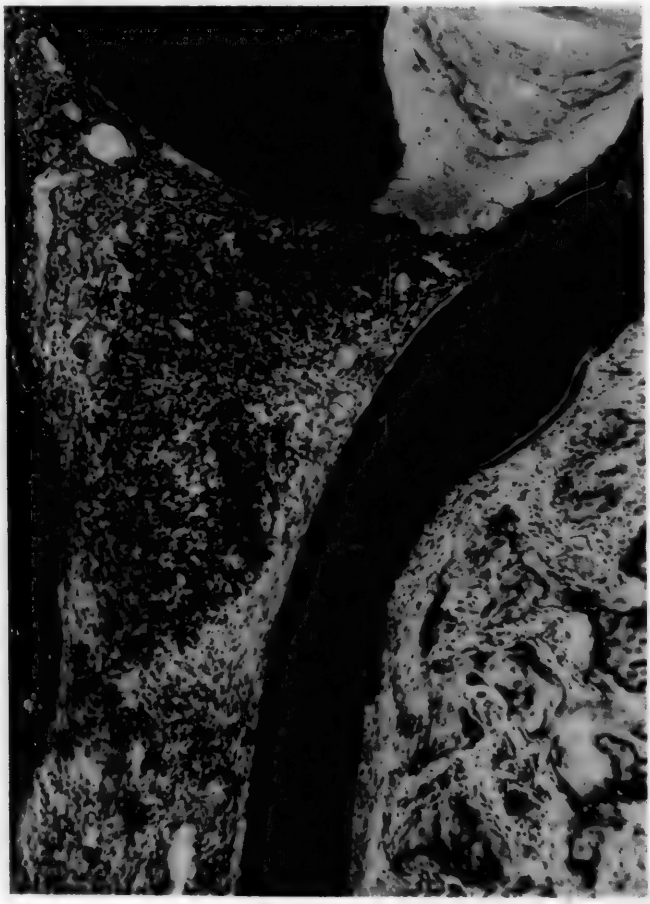

Fig. 6.—Photomicrograph showing an infiltration of polymorphonuclear leukocytes and red blood cells within the pulp under zinc oxide and eugenol 7 days after surgical amputation (Hematoxylin and eosin; Orig mag. ×80).

were observed under zinc oxide and eugenol they appeared to be associated with an infiltration of red blood cells into the tissue at 7 days after amputation (Fig. 6). By 14 days, the number of polymorphonuclear leukocytes was greatly reduced and at 21 days, they were no longer evident.

At 14 days it became clear that the basic pattern of healing was progressing in a parallel fashion below both medicaments. Specimens covered

with calcium hydroxide showed a well-formed primary calcific bridge. Some specimens were well advanced in the formation of the permanent dentin bridge. The pattern of healing in pulps covered with zinc oxide and eugenol appeared to be slightly delayed. At 14 days the primary calcific bridge was only beginning to form. The pulp tissue between the primary calcific bridge and the medicament now showed evidence of degeneration with an increased prominence of the intercellular matrix.

By 21 days the pattern of pulpal healing under both medicaments was approximately the same. Under calcium hydroxide, the permanent dentin bridge was well established. Under zinc oxide and eugenol, the dentin bridge was also well formed. Apparently, the cells under the zinc oxide and eugenol had caught up with those under calcium hydroxide and the histologic picture was the same in both groups of specimens (Figs. 1 and 4).

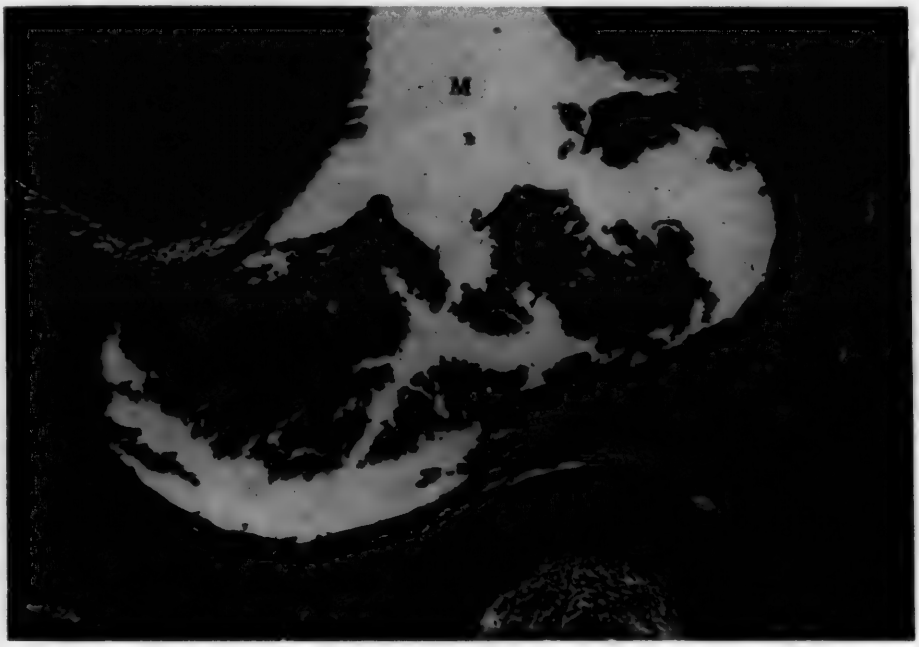

Fig. 7.—Photomicrograph showing a delayed pulpal reaction 7 days after surgical amputation and covering with zinc oxide and eugenol (Hematoxylin and eosin; Orig. mag. ×80). Note large pool of whole blood overlying amputated pulp surface. This has retarded rate of pulpal healing. There is no coagulation of tissue nor zone of degeneration yet evident. *M*, area of medicament lost during sectioning.

At 28 days, it was difficult to distinguish between amputated pulps treated with calcium hydroxide or zinc oxide and eugenol. The exposure under each medicament was sealed off by a newly formed dentin bridge. The pulps were normal and there was no evidence of chronic inflammation under the calcium hydroxide or the zinc oxide and eugenol.

Although some differences did exist at 7 and 14 days between specimens covered with calcium hydroxide or zinc oxide and eugenol, these differences

were no longer apparent at 28 days. This suggests that the pulp has a pattern of healing which can be accelerated or retarded to some degree but which cannot be altered basically by the medicament used.

Effects of Blood on Pulpal Healing.—

In many specimens a broad layer of blood (whole or hemolyzed) or exudate intervened between the medicament and the pulp. In these specimens, the pulpal reaction was obviously related to this covering rather than to the medicament.

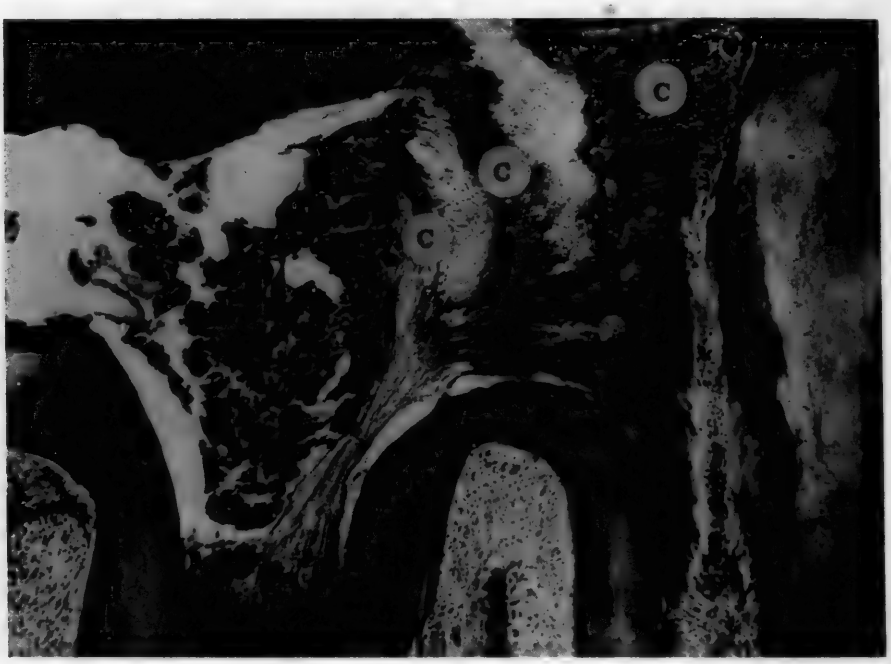

Fig. 8.—Photomicrograph showing the effect of the loss of filling material (Hematoxylin and eosin; Orig. mag. ×80). Note the 3 zones of diffuse dystrophic calcifications (*C*). Alternating zones probably indicate successive attempts by pulp to seal off exposed area but contamination has resulted in pulpal degeneration.

When a layer of whole blood (not coagulated or clotted) covered the amputated pulp, the effect was to retard the degeneration of the underlying tissue and thus also to delay the appearance of the primary and permanent dentin bridge. This appeared more often under zinc oxide and eugenol (Fig. 7).

Under calcium hydroxide, when a layer of blood intervened between the medicament and the pulp, the blood cells were coagulated (Fig. 3). In such specimens, the rate of healing appeared to be somewhat accelerated.

In no case was clotting or organization of the blood observed. When blood was present over the pulp, it remained throughout as a pool of whole or hemolyzed but unorganized blood, depending on the medicament used. The reason for the failure of the blood to clot and become organized is not clear.

It was clear, however, that the presence of such a pool of blood generally slowed down the pattern of healing probably by interfering with the action of the medicament on the pulp itself. This was seen more often under zinc oxide and eugenol than under calcium hydroxide since the latter tended to coagulate the blood and thus to accelerate the rate of healing.

Effect of Loss of Fillings.—

In specimens from which the fillings were lost or when marginal leakage had occurred, healing did not take place. In these, extensive pulpal degeneration invariably followed.

It was possible to determine approximately when the filling had been lost by measuring the amount of secondary dentin formed along the dentin walls adjacent to the site of amputation and by observing to what stage the pattern of healing had progressed. Many pulps, although exposed to oral fluids, still attempted to seal off the exposure by the formation of a wide zone of dystrophic calcification (Fig. 8). This demonstrated the great recuperative powers of the pulp and emphasized (to us) the important fact that the success or failure of bridging under a pulpotomy depends more on the integrity of the filling and the adequacy of the marginal seal, than upon the substance used to cover the amputation.

DISCUSSION

Stages in Pulpal Healing.—This study emphasizes the fact that each stage in the healing process is dependent upon the presence and character of the preceding morphologic zone (Fig. 9). It was seen that calcium hydroxide coagulates and necrotizes the superficial pulp tissue as described by Glass and Zander.[9] This appeared to stimulate the rapid formation of the primary calcific bridge. On the other hand, zinc oxide and eugenol did not cause necrosis of the superficial pulp tissue and degenerative changes were delayed. This resulted in a comparatively late formation of the primary and the permanent dentin bridges.

The basic reaction observed during pulpal healing was a protective walling off of the site of injury by a fibrous calcification of the peripheral portion of the degenerating pulp at its junction with the vital pulp tissue (the primary calcific bridge). This temporary bridge allowed the vital portion of the pulp to organize its cellular elements toward the formation of a more permanent seal, the permanent dentin bridge.

The formation of a primary calcific bridge represents the primary defensive reaction of the degenerating but still vital pulp cells. O'Malley[6] referred to this as an area of dystrophic calcification while Fish[8] called it a true calcific degeneration. It is interesting to speculate as to the source of the fibers and the calcium salts necessary for the calcification of this zone. Are the fibers a product of the degenerating cells (fibroblasts?) entrapped in the area or are they precipitated or polymerized from the protein matrix in the area? Do the calcium ions come from the underlying vital pulp or from the

medicament above? Or are the calcium salts contained within the fibers and cells in the area, being precipitated during the process of "calcific degeneration"? This study indicates that the calcium is not derived from the medicament above, else the primary calcific bridge would not be formed under zinc oxide and eugenol.

The primary calcific bridge may be regarded as the organizer of the healing process in that it is always seen prior to the formation of the permanent dentin bridge. It has been postulated that a calcified material must be present to provide a stimulus for the differentiation of new dentin-forming cells. It was observed that a thin zone of dystrophic calcification leads to the early formation of a thick permanent bridge while a wide diffuse primary bridge leads to a thin and late permanent bridge. This relationship was also noted by Miyamoto.[7]

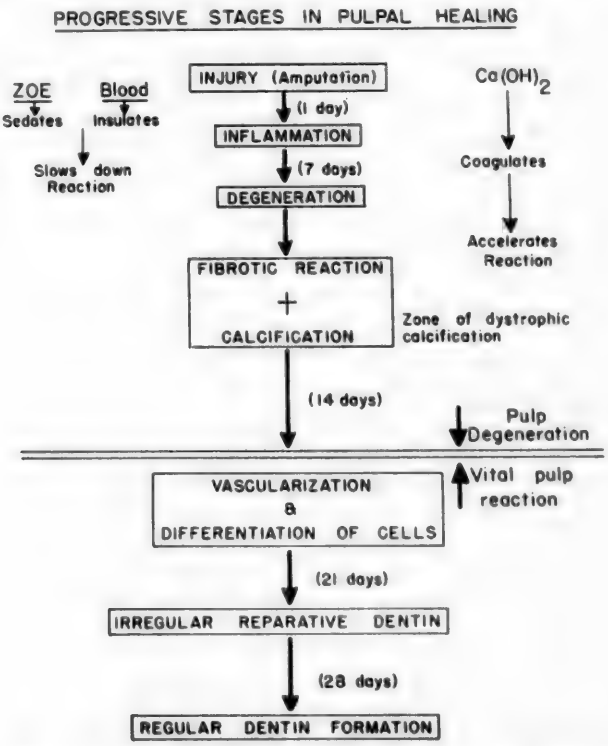

Fig. 9.—Diagram showing progressive stages in pulpal healing.

The Permanent Dentin Bridge.—The term "permanent dentin bridge" is used here to describe a dentinlike material formed by vital cells (odontoblasts or odontoblast-like cells) which reaches from one lateral margin of the exposure to the other. A question that has often been raised is the source of the cells that form the permanent dentin bridge. The findings in this investigation suggest that both old cells, which have migrated from the dentinal walls, and new cells enter into the formation of the dentin bridge. It was observed that

the quickly formed dentin bridges under very small exposures were laid down primarily by surviving odontoblasts from the lateral walls near the exposure. Under large exposures, the main portion of the bridge was formed by newly differentiated cells aided by old odontoblasts lining the lateral dentin walls.

Effect of Medicament.—The findings in this investigation indicate that healing of surgically amputated pulps takes place below zinc oxide and eugenol as well as under calcium hydroxide. The pattern of healing, as described in this paper, does not support the thesis that an exogenous calcium salt is essential to pulpal healing or bridging of the amputation site. The most important evidence is the fact that (a) bridging can and does take place under noncalcium materials in direct contact with the pulp, and (b) calcification of the primary bridge takes place at an area farthest from the medicament and at the junction of the vital pulp tissue. All the evidence points to the vital pulp tissue as the "reactive" zone and the source of the calcium ions necessary for calcification of both the primary and permanent bridges.

Minor differences were observed between the pulpal reactions under calcium hydroxide and under zinc oxide and eugenol. These differences were confined entirely to the first 14 days and were related to the rate of healing—not the type or quality of healing. It is clear that the differences were the result of the effect of these substances upon the superficial layer of the pulp only (Fig. 9). Once the superficial zone had degenerated (after 14 days) and the medicament was separated from the underlying vital pulp tissue by a wide zone of degenerated and calcified material, the influence of the medicament disappeared or at least became much less apparent.

Calcium hydroxide had a necrotizing effect on the contiguous pulp tissue or blood. Characteristically, the pulpal reaction below this coagulum proceeded more rapidly than under the relatively less injured tissue covered by zinc oxide and eugenol. These observations with reference to calcium hydroxide have been reported previously.[9]

This study showed that many (but not all) of the pulps capped with zinc oxide and eugenol had an aggregation of polymorphonuclear leukocytes in the superficial pulp tissue at 7 and 14 days after amputation. This confirms the findings of Glass and Zander[9] and O'Malley.[6] However, pulps examined 21 and 28 days following amputation did not show a persistence of these inflammatory cells.

Loss of Filling.—This study re-emphasized the common clinical observation that pulpal healing will not take place if the filling is lost or leakage occurs. This factor, more than any other investigated in this study, determined whether or not the pulp would heal.

SUMMARY AND CONCLUSIONS

Surgical amputations were performed on the pulps of 122 maxillary first molars of 61 albino rats. Half of the amputated pulps were covered with calcium hydroxide and half with a zinc oxide and eugenol paste. The animals were sacrificed at 7, 14, 21, or 28 days and the teeth subjected to histologic analysis for the purpose of studying the pattern of pulpal healing.

It is believed that a suitable technic has been developed for performing pulpotomies on the first maxillary molars of the rat. The method is reproducible and may be used for further investigations into the effects of age and various medicaments upon pulpal healing following surgical amputation. For these studies, the rat molar is a suitable biologic test object.

1. In the rat molar a stereotyped pattern of healing was observed after surgical amputation of the pulp. This pattern was basically the same under any of the medicaments used.[7] It was characterized by the formation of a superficial zone of pulpal injury and degeneration under which a layer of dystrophic calcification was formed followed by the building of a true dentin bridge. The primary calcific bridge appeared to be directly related to, and essential for, the differentiation of new odontoblasts and the formation of a true dentin bridge. The outstanding characteristic of the healing pattern was that the formation of each zone was dependent upon the presence and character of the layer above (Fig. 9).

2. Although differences in the pulpal reactions under calcium hydroxide and zinc oxide and eugenol could be observed at 7 days after amputation, by 21 days the histologic picture was approximately the same. Calcium hydroxide is therefore not essential to pulpal healing and bridging. However, calcium hydroxide caused a more rapid necrosis and degeneration of the superficial layer of pulp tissue and a more rapid appearance of the primary calcific bridge. The action of the calcium hydroxide in promoting healing may be on the basis of its high alkalinity and coagulating action rather than on its calcium content since healing also occurred under zinc oxide and eugenol albeit at a slower rate.

3. Unorganized blood seemed to retard healing while precipitated or coagulated protein material (pulp tissue or exudate) appeared to favor pulpal healing.

4. A prime requisite for pulpal healing appeared to be an adequate seal against the ingress of oral fluids. This factor overshadowed all others. In this study, the medicament used did not appear to be the major factor in promoting the bridging of the exposure.

The authors wish to thank W. M. Winn for making the photomicrographs.

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STUDY OF THE SUBMICROSCOPIC STRUCTURE OF HUMAN DENTAL ENAMEL BY ELECTRON MICROSCOPY

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THE technics of x-ray diffraction and polarized light have been used in a number of studies of the structure of mature human enamel.[1-4] These methods have provided (a) a rough determination of the size of the enamel crystals, (b) an estimate of the mean orientation of crystals with respect to the prisms, and (c) proof that the inorganic material consists of hydroxyapatite. These methods do not permit direct measurement of the physical dimensions of the crystals nor can irregularities in their shape be detected. This information should be obtainable by the electron microscope and although many workers have been interested in this field,[5, 6] their results have not been conclusive. Very thin sections of demineralized enamel have been used to demonstrate that the submicroscopic fibrillar network is the smallest organic structure present in the matrix of mature enamel,[7] but this sectioning technic does not provide any information on the crystal size or shape.

It was felt that the use of high resolution replicas of fractured enamel surfaces would yield more accurate information than those of polished and etched surfaces, particularly with regard to the details of the crystals in situ. This method has been used elsewhere to study the structure of enamel, but Helmke is the only worker who has used it extensively.[8, 14] His research, however, has been concentrated mainly on the orientation of the crystals with respect to the prism rather than details of the crystals themselves.

In this study of fractured enamel surfaces we found it possible to observe the shape and size of the crystals, the relationship of the crystals to the submicroscopic organic matrix in different areas of the teeth and, to a more limited extent, the orientation of the crystals in the prisms and the so-called interprismatic areas. We have also been able to show that the organic structure is somewhat more complex than that described by Scott.[7, 9, 10]

SPECIMEN PREPARATION

The teeth used for the studies were mainly premolars of children between the ages of 10 and 14 years. These teeth were vital and noncarious and, after removal for orthodontic reasons, were fixed in ethanol for a period of approximately 2 days. Both erupted and unerupted teeth from patients in the 17- to 20-year-old group were also used and a number of these teeth were fixed in 1 per cent osmic acid-veronal buffer.[11]

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The carbon replicas were prepared for electron microscopy from fractures of teeth, by the method previously described.[12] The prints of the micrographs have been made from the negatives and all shadows appear white. It has not been possible to show fine detail as clearly on the prints as it can be observed from the negatives with the aid of a microscope. In Figs. 1, 3, and 4 a contrast control process was used to show the detail.[13]

OBSERVATIONS

The general appearances obtained from replicas of fractured surfaces are shown in Figs. 1 and 2. Fig. 1 was obtained from a fracture of the cervical region of an unerupted third molar, while Fig. 2 was obtained from a fracture of the cuspal region of an erupted premolar. In both figures, the areas marked *P* represent prismatic regions, while the areas *IP* are probably oblique fractures of prismatic regions, although in some cases they may be interprismatic regions. It has not always been possible by this method to determine into which category the areas *IP* belong but in Fig. 2 it appears that these areas are almost certainly not interprismatic regions.

In Fig. 1, the enamel has been fractured oblique to the direction of the prism crystals and many opaque fragments of crystals attached to the replica are visible. Some of these lie in the same general direction as that of the replicated crystals.

In Fig. 2 the areas marked *P* were fractured in a direction nearly perpendicular to the direction of the prism crystals. More or less circular areas, having an average diameter of 170 mμ, fill the prism face and the number of these across the width of the prism varies from 20 to 25. Each of these small areas has finer detail and is surrounded by a sheath which is probably organic. This appearance is shown in greater detail in Fig. 8, which will be discussed later.

Again, in Fig. 2, it is apparent that in the areas marked *P* the loose crystals have broken off in rounded fragments while, in the areas marked *IP* where the crystals are fractured longitudinally, the loose crystals tend to lie lengthwise on the replica. In some of the more readily interpreted micrographs these crystals appear to lie at an angle of not less than 30 degrees with respect to the long axis of the prism. However, the exact angle of fracture cannot be determined from replicas of fractures.

From observations of such fractured surfaces at a magnification of ×20,000 it became apparent that there were differences in the microstructure of the enamel from the cervical and the cuspal regions of freshly erupted teeth. An attempt was made, therefore, to determine the relationship of the organic network to the inorganic crystals in these regions.

Enamel From the Cervical Region in Freshly Erupted Teeth.—A large number of micrographs of fractures of alcohol-fixed teeth were reviewed and Fig. 3 is representative of one of these taken from the cervical region. In this figure, in areas similar to *A*, the crystals are replicated longitudinally and rows

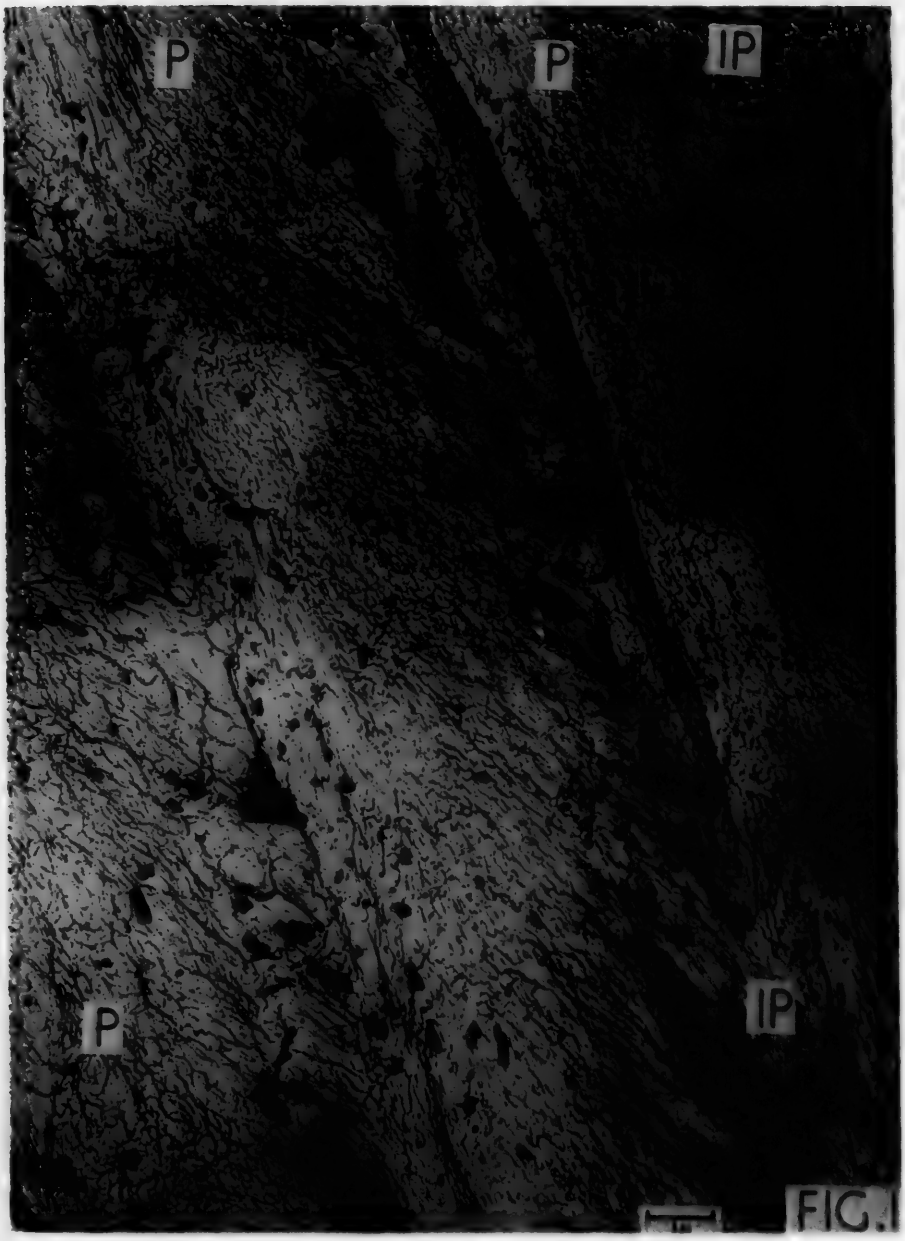

Fig. 1.—Replica of a fracture of cervical region of unerupted third molar, showing prismatic regions.

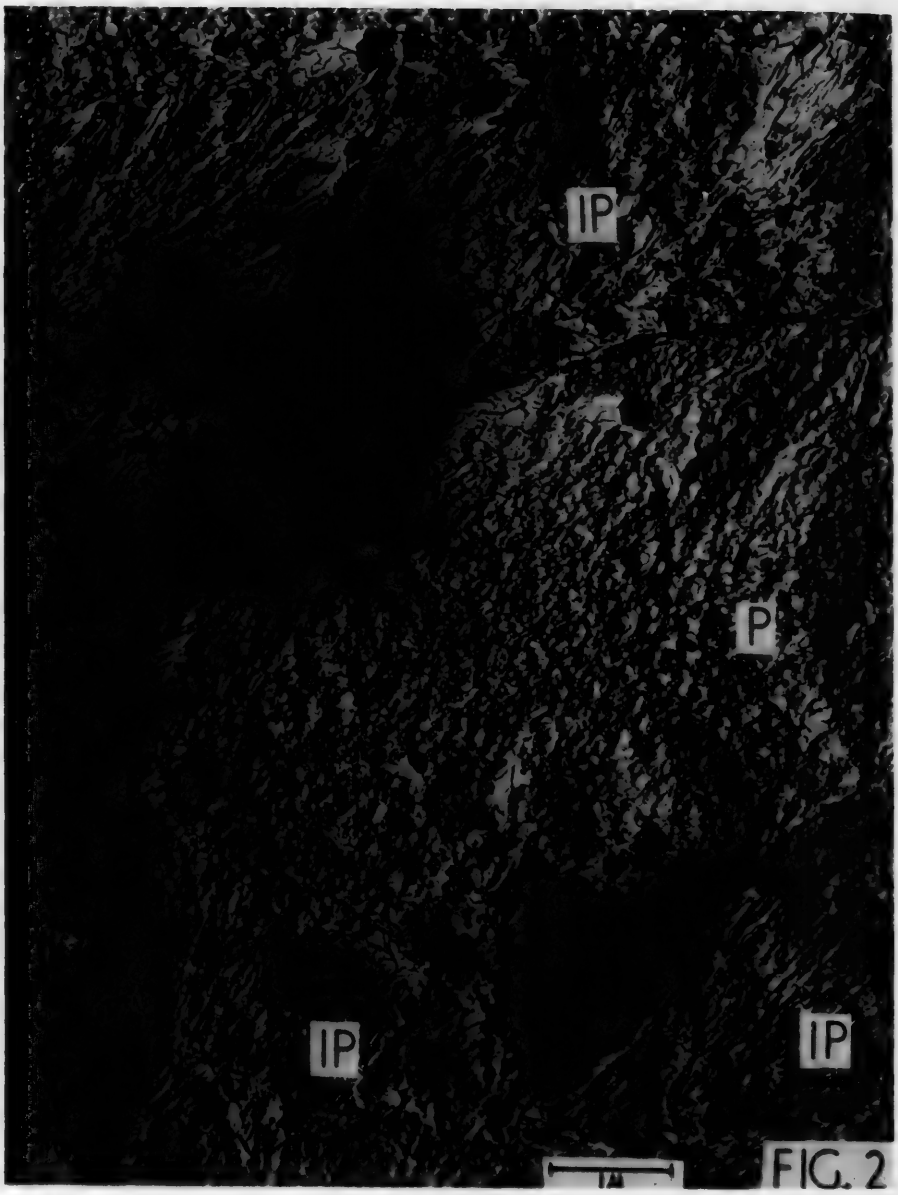

Fig. 2.—Replica of a transverse fracture of cuspal region of erupted premolar showing cross section and side view of crystals.

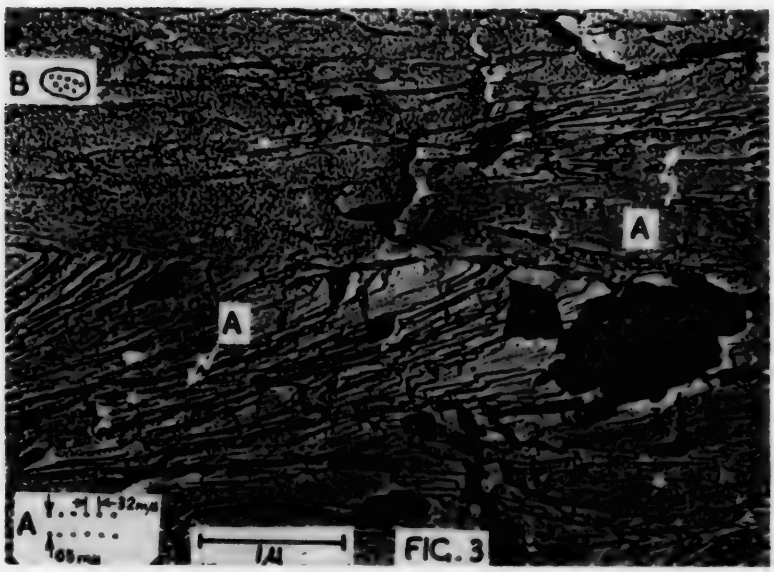

Fig. 3.—Replica of cervical region alcohol-fixed tooth at higher magnification than Fig. 1, showing periodic detail in organic and crystal structure.

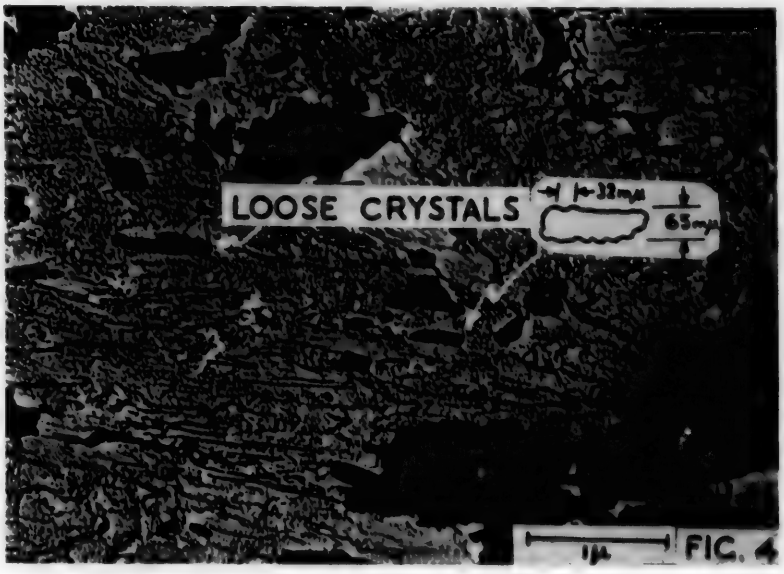

Fig. 4.—Serrated edge of loose crystals from a longitudinal fracture.

of small dots are visible with a periodic spacing of 32 mμ, and loose crystals can be observed lying between the rows. It is suggested that these small dots are of organic material, since loose crystals have been observed having serrations with a similar periodicity. Some loose crystals showing this are seen in Fig. 4. The areas marked as *B* in Fig. 3 show the detail apparent in a transverse fracture of crystals.

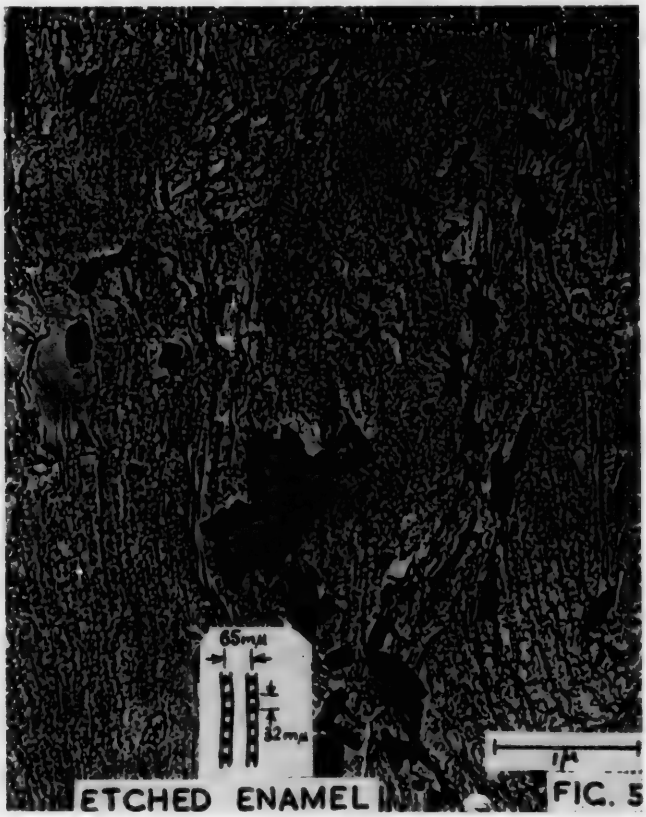

Fig. 5.—Replica of partially demineralized enamel showing periodic structure in organic fibers.

The use of a fixative of 1 per cent osmic acid-veronal buffer for a period of 48 hours appears to make the organic fibrillar material stand out in greater detail. The appearances seen with this fixative are shown in Figs. 5 and 6. Fig. 6 represents a replica of a longitudinal fracture and shows a number of long organic fibers which have remained attached to the replica.

These fibers present 2 interesting features. Some of them are split open and these appear to have double walls with a hollow center. The space between the inner and outer walls is occupied by an internal structure which divides the fiber transversely at intervals of 16 to 18 mμ. The second feature of interest is the appearance of small cups distributed along and around the

fibers at intervals of 32 mμ. These cups give the fiber the appearance of the tentacles of a squid. The fibers have been checked in a through-focus series to determine whether the center of the cups merely appeared hollow due to under-focus fringes but the result of this examination shows that the appearance is genuine. In the insert of Fig. 6, the appearance is shown diagrammatically and three cups are shown spaced around the circumference but the exact number is not definitely known.

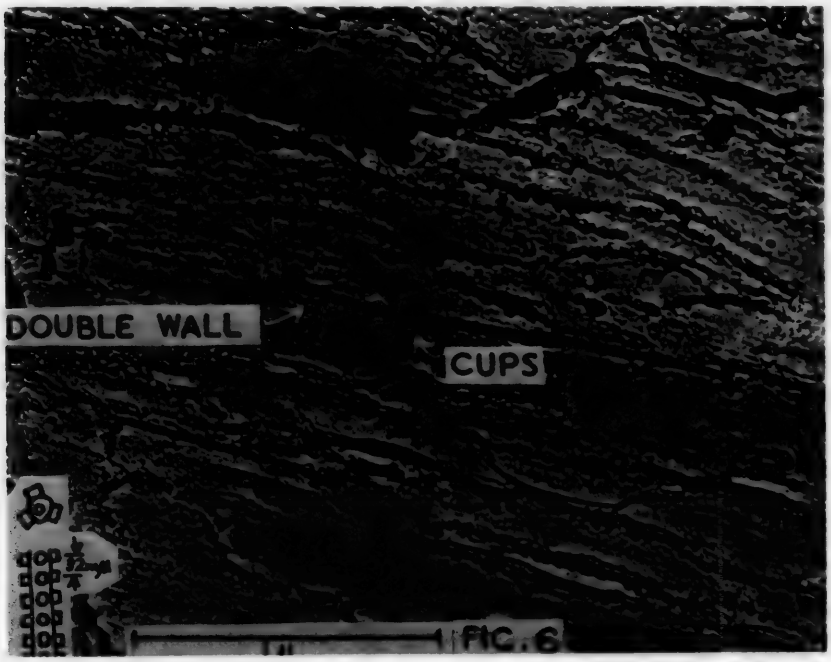

Fig. 6.—Showing detail of organic fibers attached to replica. Obtained from surface treated with osmic-acid veronal buffer.

The distance, as measured between a large number of these longitudinal fibers, has a mean value of 65 mμ and, as this corresponds to the mean width of the loose crystals, it seems that these fibers determine the width of the crystals in the enamel of the cervical region of freshly erupted teeth. In other words, in this enamel region the organic framework tends to enclose individual crystals rather than groups of crystals.

Cuspal Enamel.—In examining the cervical enamel it was found that the length of the crystals could not be readily estimated but later an approximate value was obtained from replicas of fractures of enamel near the crown. The appearance of these replicas is shown in Figs. 7 and 8. From these a mean value of 0.4 μ has been estimated for the length of the crystals. However, there is a considerable range about this value, limits being about 0.1 μ to 1.0 μ.

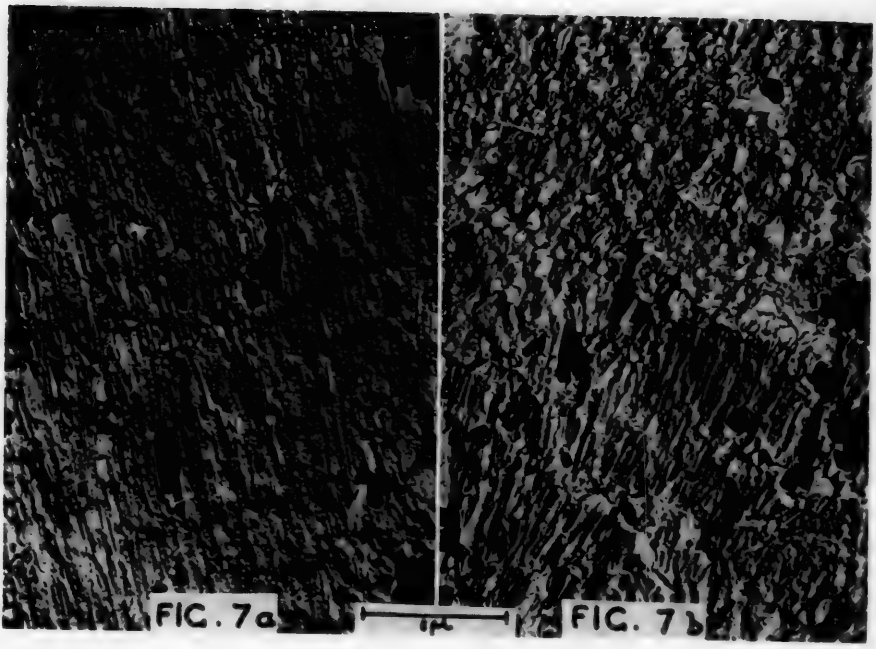

Fig. 7.—From same micrograph as Fig. 2, enlarged to show minute dents and bumps.

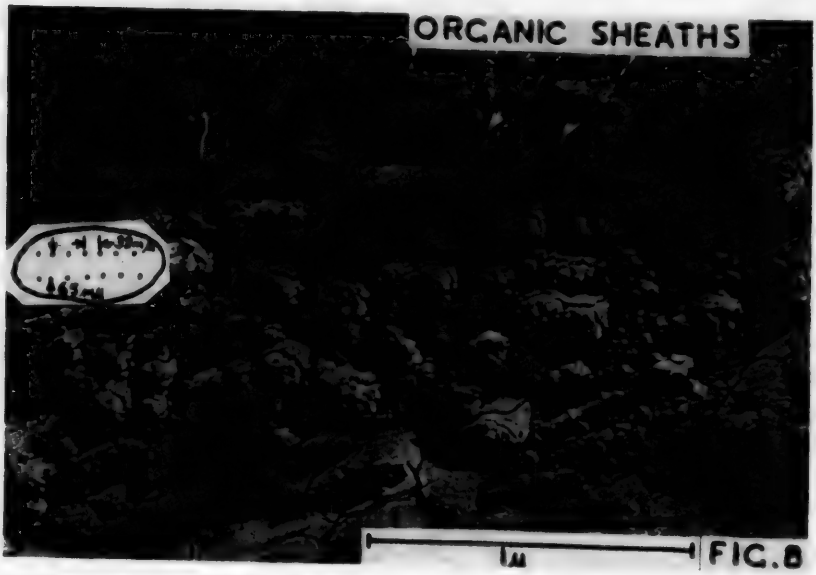

Fig. 8.—Fracture from cuspal region of premolar showing what appears to be mainly inorganic minute projections along crystals.

In certain areas of Figs. 3, 5, and 6, the longitudinal fibers are packed closely together. It is felt that in these areas the crystals are lying edgewise but due to the thickness of the organic fibers they are not visible. An attempt was therefore made to estimate the thickness of the crystals from the length of the shadows of loose crystals. It is difficult to do this with any great degree of accuracy because the replica is not always flat but an estimate of 10 mμ is considered to be an average value. The resolving power of our instrument, which is 50 Å, does not permit of direct observation of the crystals as they would appear end-on in transverse fractures such as Fig. 7, *B*.

Fig. 7, *A* is a magnified area of Fig. 2 and shows many minute hollows and a few corresponding elevations where the crystals lie flat. These little dents and bumps can also be seen in the transverse fracture in Fig. 7, *B*. A similar appearance can be seen in Fig. 8 and in this figure they show a definite appearance of consisting of inorganic material. When measured along the crystals, these hollows have a spacing of 32 mμ which is the same spacing as that observed in the organic fibers seen in Fig. 3 from enamel from the cervical regions. From this observation, therefore, it would appear, at least in the cuspal regions, some of the organic fibers become condensed and the inorganic material takes up the original spacing and dimensions of the organic fibers. It is not known whether this occurs during the so-called maturation of enamel or whether it is a slow process taking place over a period of several years after the tooth has erupted.

In the cervical enamel, the average spacing between the longitudinal fibers was 65 mμ and corresponded to the width of the crystals. In the cuspal enamel, however, the spacing between the organic fibers has increased to 170 mμ and these fibers now appear to enclose a number of crystals. When viewed in cross section as in Fig. 8, the number of crystals thus enclosed varies from 1 to 8, with an average of between 2 and 3. In some micrographs there is a suggestion that these crystals show a more detailed structure but further work with a modern electron microscope would be necessary to confirm this.

DISCUSSION

The investigation of human dental enamel was prompted by interesting replicas of enamel which were obtained during recent electron microscopic studies of dentin.[14] During this study a difference was detected between the cuspal and cervical enamel in freshly erupted premolars. It appeared advisable to determine 2 things: (1) whether the submicroscopic fibrillar network which we observed was identical with that described by Scott,[7, 9, 10] and (2) the reason for the appearance of serrated edges on loose crystals of enamel.

The results of this study show that the organic network observed by Scott, after sectioning demineralized mature enamel, is identical with that obtained from replicas of fractures of cuspal enamel (Figs. 2 and 8), if we use an instrument with a resolving power of not better than 50 Å. When replicas of a similar nature are observed with an instrument with a greater resolving power it is considered that a finer organic structure will be evident, showing that the organic fibers evident in the cervical enamel have become condensed.

In the photomicrographs of replicas of fracture surfaces, the network of the surface enamel contains 20 to 25 openings in the width of a prism and each opening contains a number of crystals. From the dimensions which have been given it is possible to stack up to 30 crystals in each of these openings when viewed transversely. As the width of each crystal is 65 mμ and the thickness of the fibers approximately 10 mμ, then, allowing 1 fiber for every 2 crystals (Fig. 8), the number of crystals per prism width lies between 60 and 425. Confirmation of this could be obtained by the observation of transverse fractures with an electron microscope possessing a resolving power of the order of 10 Å.

The appearance of the cups distributed longitudinally on the fibers (Fig. 6) led to the suggestion that these cups may be the points of origin of the precipitation of the inorganic salts for the enamel crystals. It would also appear that the inorganic material which subsequently replaces some of the organic material in the majority of the fibers and forms the small dents and bumps seen in Figs. 7 and 8, also originates from these same points. The material which occupies the space left by the condensed fibers is presumably hydroxyapatite but this is not necessarily so. No evidence has been obtained from this study as to whether this material has the same solubility in acid as hydroxyapatite.

As the cervical enamel contains at least 50 per cent more organic fibers than the cuspal enamel then it is logical to believe that it must be more resilient. Furthermore, in the cuspal enamel when the inorganic material has replaced in part the organic fibers, then the crystals become keyed to each other and logically this would prevent the crystals sliding on the surface of each other, especially if the constraining force of the remaining organic material is appreciable. These facts may have an important bearing on the ability of the enamel to withstand the forces to which it is normally subjected without distortion, fracture, or detachment from the underlying dentin.

CONCLUSIONS

1. The enamel crystals have a width of 65 mμ, a length which varies widely but has a mean of 0.4 μ and a thickness which is estimated to be 10 mμ.

2. The edges of crystals from the cervical region of recently erupted teeth are serrated in a manner similar to that of a postage stamp.

3. The serrations are due to small organic projections on the sides of the organic fibers and have a periodicity of 32 mμ.

4. At least half of the organic material in the fibers which are present in young enamel are replaced by inorganic material as it becomes more mature.

5. The organic network observed by the Metropolitan Vickers EM2 microscope in a transverse fracture of cuspal enamel is identical with the submicroscopic network observed by Scott.

6. The number of crystals contained in each submicroscopic sheath is between 20 and 30.

7. The dimensions of the crystals are determined by the organic fibers which, in the cervical enamel, form the boundaries of each crystal.

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SOLUBILITY RATE AND NATURAL FLUORIDE CONTENT OF SURFACE AND SUBSURFACE ENAMEL

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THE surface enamel is more resistant to acid than is subsurface enamel, the rate of dissolution of phosphate from the surface being less than that from the subsurface enamel.[1-3] With ordinary and polarized light microscopy, as well as with soft x-rays, it has also been shown that it is possible for demineralization of the subsurface enamel to occur without any apparent change in the enamel surface,[4, 5] and when ground sections of teeth were exposed to EDTA the outer portion of enamel was the last to be demineralized.[6]

The reason for this difference between surface and subsurface enamel has not been established. That fluoride may play a role, at least at the level of 2 ppm in the water supply, is suggested by the work of Jenkins, Armstrong, and Speirs,[2] who found that the solubility rate of surface enamel of teeth from areas with 2 ppm fluoride in the water supply was less than that from areas with 0.25 ppm, although there was no difference in the solubility rate of the bulk of the enamel. Since fluoride accumulates in the surface enamel, even in teeth from areas low in fluoride,[7] it was considered that fluoride acquisition might generally be responsible for the decreased solubility rate of surface compared to subsurface enamel. Therefore, in the present study an attempt was made to correlate the solubility rate and fluoride concentration of successive layers of enamel from areas with different levels of fluoride in the drinking water.

MATERIALS

Samples of 6 successive layers of enamel obtained for a previous study[7] were sufficiently large to provide aliquots for fluoride analyses as well as for solubility tests. The enamel was ground from teeth of persons who were born and were living in cities where the water supply contains 0.1, 1.0, or 5.0 ppm fluoride. Samples from about 40 teeth in each of the 8 groups described in Tables I, II, and III were used in this study. The enamel samples all passed through a 270-mesh sieve. The approximate distribution of large and small particles below a 270-mesh level as gauged by microscopic examination appeared to be similar in all samples.

METHOD

The enamel samples were dried at 110° C. to constant weight. A 50 mg. portion from each sample was placed in a centrifuge tube of known weight and exposed for 1 hour at room temperature to 5 c.c. of 0.5 M acetate buffer at pH

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TABLE I

ENAMEL FROM DECIDUOUS, UNERUPTED, AND ERUPTED PERMANENT TEETH OF PERSONS UNDER 20 AND OVER 50 YEARS OF AGE, LIVING IN AN AREA WITH 0.1 PPM F* IN THE WATER SUPPLY†

	DECIDUOUS			UNERUPTED			UNDER 20		OVER 50	
		% WEIGHT LOSS			% WEIGHT LOSS			% WEIGHT LOSS		% WEIGHT LOSS
LAYER	F	pH 4	pH 5	F	pH 4	pH 5	F	pH 4	F	pH 4
1	240	40.0	21.1	616	36.5	18.8	460	37.6	1,080	35.7
2	129	43.8	22.6	257	41.5	19.8	114	41.2	540	41.8
3	55	48.1	24.0	143	41.0	21.1	93	42.3	305	44.4
4	54	48.0	24.7	100	42.9	20.5	Lost		190	46.0
5	49	48.8	23.8	70	45.0	21.0	51	45.4	198	48.8
6	50	48.6	24.6	52	45.4	21.7	—	47.4	114	48.1

*Chicago, Ill.

†Fluoride content in ppm and percentage loss of weight from 50 mg. samples of successive layers of enamel exposed to 5 c.c. of 0.5 M acetate buffer at pH 4.0 or pH 5.0 for one hour.

TABLE II

ENAMEL FROM ERUPTED PERMANENT TEETH OF PERSONS UNDER 20 YEARS LIVING IN AREAS WITH 0.1 PPM F* OR 5.0 PPM F† IN THE WATER SUPPLY‡

		0.1 PPM F IN WATER % WEIGHT LOSS			5.0 PPM F IN WATER % WEIGHT LOSS	
LAYER	F	pH 4.0	pH 4.5	pH 5.0	F	pH 4.0	pH 5.0
1	499	32.5	24.3	17.0	3,370	26.8	15.0
2	162	37.6	28.8	19.7	1,710	32.9	17.0
3	108	38.6	30.3	19.8	1,124	35.9	18.9
4	63	41.4	30.8	20.8	924	38.0	19.2
5	76	42.9	31.5	21.4	811	39.3	20.3
6	42	44.3	32.5	21.6	570	40.2	20.3

*Buffalo, N. Y. and Chicago, Ill.

†Post, Texas.

‡Fluoride content in ppm and percentage loss of weight from 50 mg. samples of successive layers of enamel exposed to 5 c.c. of 0.5 M acetate buffer at pH 4.0, 4.5, or 5.0 for 1 hour.

TABLE III

ENAMEL FROM ERUPTED PERMANENT TEETH OF PERSONS OVER 50 YEARS LIVING IN AREAS WITH 0.1 PPM F* OR 1.0 PPM F† IN THE WATER SUPPLY‡

		0.1 PPM F IN WATER (N. Y.) % WEIGHT LOSS		0.1 PPM F IN WATER (ILL.) % WEIGHT LOSS		1.0 PPM F IN WATER % WEIGHT LOSS	
LAYER	F	pH 5	F	pH 4	F	pH 4	pH 5
1	971	12.7§	1,080	35.7	1,552	32.8	19.7
2	512	15.8	540	41.8	727	38.4	22.3
3	320	16.1	305	44.4	328	42.7	20.9
4	297	17.9	190	46.0	227	43.1	20.7
5	194	17.5	198	48.8	219	43.3	21.0
6	150	17.7	114	48.1	158	43.8	20.9

*Buffalo, N. Y., and Chicago, Ill.

†McKinney, Texas.

‡Fluoride content in ppm and percentage weight loss of successive layers of enamel exposed to 5 c.c. of 0.5 M acetate buffer at pH 4.0 or pH 5.0 for 1 hour.

§Figures in this column are not comparable to other groups. Samples were heat-treated (see text).

4.0, 4.5, or 5.0. A stream of air was bubbled slowly through the mixture to ensure constant agitation. After centrifugation for 5 minutes, the supernatant was removed by suction and the residual enamel was washed successively with two 5 c.c. aliquots of distilled water. The tube plus the residual enamel was dried overnight at 110° C. and the amount of enamel dissolved was determined by the loss of weight. The maximum change in the pH of the buffers was 0.2 pH unit. All tests were run in duplicate.

Nine tests on 50 mg. portions of pooled enamel showed that the average weight of enamel dissolved at pH 4.0 was 21.4 mg. with a standard deviation of 0.212. The amount of fluoride dissolved during the tests was determined by fluoride analyses[8, 9] of the enamel samples before and after exposure to acid. The quantity of fluoride present in the acid at the end of the experiment was also measured.

It was considered possible that differences in the relative organic content of the surface and inner enamel may influence their solubility rate. Therefore, in an additional experiment, the organic constituents of aliquots of enamel from a first and sixth layer were removed by refluxing with ethylenediamine, according to the method of Williams and Irvine,[10] and the solubility rate in acetate buffer pH 4.0 of the enamel thus treated and of the untreated enamel was compared. As a check on our solubility method the supernatants in this experiment were analyzed for calcium[11] and phosphorus.[12]

RESULTS

The loss of weight from the samples of successive layers of enamel on exposure to the buffer (pH 4.0, 4.5, and 5.0) is given in Tables I to III for the 8 groups of teeth studied. An increase in the weight of enamel dissolved from the first layer to the sixth layer was noticed in all the groups at all pH levels. The difference between the loss of weight from the first and second layers generally was most marked. The probability of this difference occurring by chance was less than 0.05. Although the differences between the deeper layers were less, there was a consistent trend toward a gradual increase in weight loss with depth.

The data given in Table II for the under 20 age group from the 0.1 ppm fluoride area are graphed in Fig. 1. Here the loss of weight from each layer at pH 4.0, 4.5, and 5.0 is plotted against its cumulative depth from the surface. An index of the depth or thickness of each layer was obtained by expressing the weight of each layer as a percentage of the sum of the weights of all the 6 layers. The weight loss of any given layer was taken to represent the weight loss at the mid-point of that layer. The resulting curves show that (1) at the 3 pH levels, the enamel surface is more resistant than the inner enamel, (2) the difference between the loss of weight from the surface and inward is most marked at pH 4.0 and least evident at pH 5.0, and (3) at pH 4.5 the loss of weight from each layer lies approximately half way between the corresponding values obtained at pH 4.0 and pH 5.0.

In Fig. 2, the loss of weight from successive layers of enamel is plotted against depth from the surface for the 7 groups tested at pH 4.0. In order to

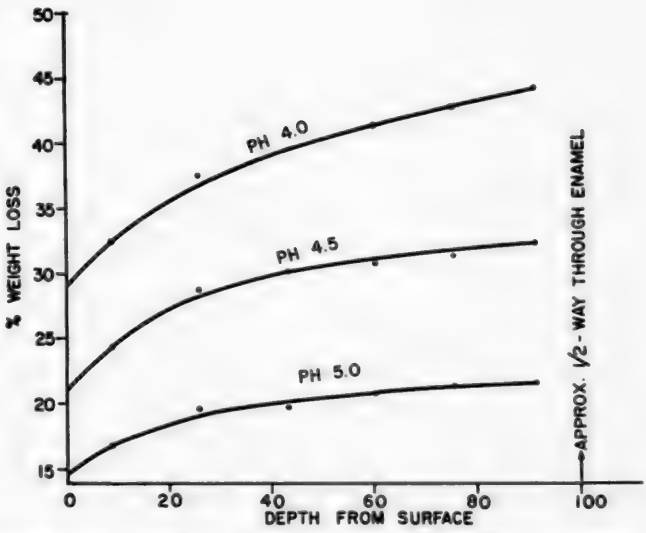

Fig. 1.—Weight loss of successive layers of enamel. Exposure to acetate buffer for 1 hr. (permanent teeth—20 years).

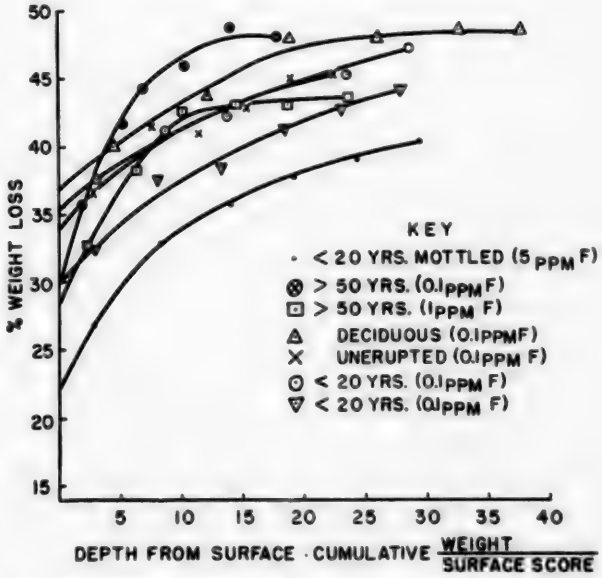

Fig. 2.—Weight loss of successive layers of enamel. Exposure to acetate buffer (pH 4) for 1 hr.

compare the groups with each other as accurately as possible, a thickness index of each of the layers ground in each group was estimated as previously described.[13] In Fig. 2, the cumulative value of this thickness index or "thickness score" of each layer is plotted as equivalent to the depth of the layer from the surface. The weight loss of any given layer was taken to represent the weight loss at the mid-point of that layer. The curves on Fig. 2 show the increase in the loss of weight from the surface inward in all groups. All the curves have a similar shape and slope except the 2 groups over 50 years of age which have a steeper rise in the early portion. Evidently there is a greater difference in the rate of solubility between the surface and subsurface enamel in teeth from older than from younger persons.

The curves of Fig. 2 also indicate that, at pH 4, less enamel was dissolved from each of the layers of the mottled group (under 20 years of age, 5 ppm fluoride area) than from the other groups, the surface enamel of the deciduous group was more soluble than the surface enamel of the permanent teeth and there appears to be no relationship between age and the solubility rate of enamel from permanent teeth.

Contrary to expectation, the weight loss at pH 5 of enamel samples from the over 50 age group from the 0.1 ppm fluoride area (Table III) appears to be much lower than the corresponding pH 5 values obtained from the 1 ppm fluoride area teeth. The 0.1 ppm area enamel had been used in a study of density in the course of which it was exposed to water and dried overnight at 110° C. 3 times; according to Sellman,[14] this treatment should decrease its solubility. Therefore, this group cannot be compared to the other groups. However, it is interesting that, although the values are correspondingly lower than in the other groups, there is the same pattern of increase in the loss of weight from the first layer inward.

On Fig. 3, the loss of weight at pH 4 from each successive layer of enamel is plotted against the logarithm of the fluoride concentration in the corresponding enamel sample. With each group, there appears to be an inverse linear relationship between the weight of the enamel dissolved by the acid and logarithm of the fluoride concentration in the enamel sample. This is true for all groups except the over 50 age groups in which the relationship is not linear. These 2 groups differed from the others in the shape of their weight loss depth curves.

It is evident that a considerable weight of enamel was dissolved by the buffer at pH 4. The question arises as whether the fluoride in enamel goes into the solution under the conditions of our experiment. The fluoride concentration in successive layers of enamel before and after exposure to the buffer is given in Table IV. In Fig. 4, these figures are plotted against depth from the surface for 2 of the groups studied. The apparent increase in the ppm of fluoride of the enamel after exposure to the buffer shows little fluoride dissolved under the conditions of our experiment. This finding was confirmed in that little or no fluoride was detected in the buffers after exposure to the enamel samples.

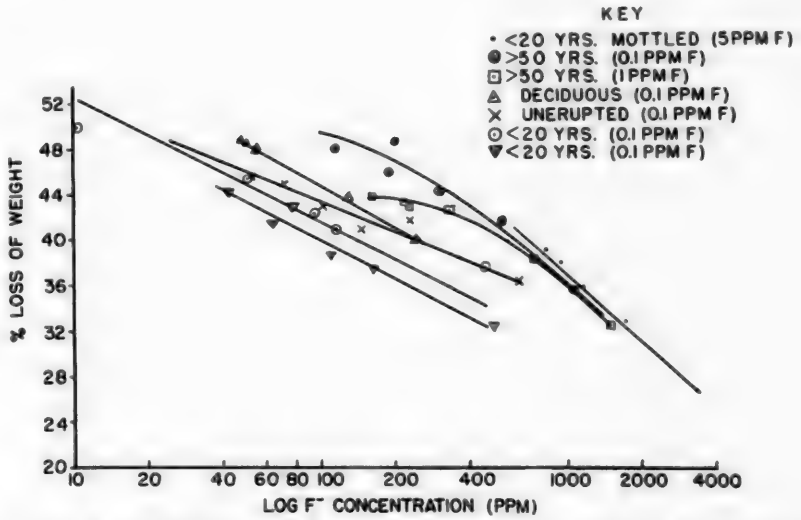

Fig. 3.—Log F^- concentration (ppm) versus solubility rate. Successive layers of enamel exposed to acetate buffer pH 4 (1 hr.).

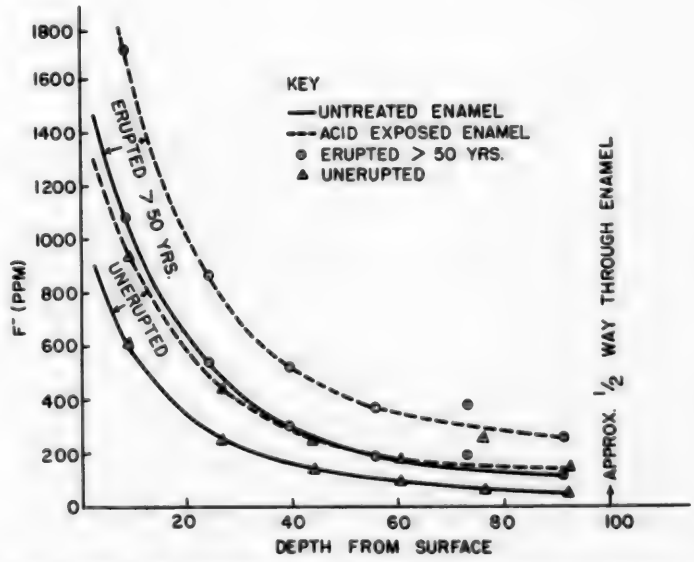

Fig. 4.—F^- (ppm) in successive layers of enamel before and after exposure to acetate buffer (pH 4) for 1 hr.

TABLE IV
PPM FLUORIDE IN SUCCESSIVE LAYERS OF ENAMEL BEFORE AND AFTER ACID EXPOSURE FOR ONE HOUR AT pH4 AND pH5

	UNERUPTED, CHICAGO*			50 YRS., CHICAGO*		50 YRS., BUFFALO*	
		ACID TREATED			ACID TREATED		ACID TREATED
LAYER	UNTREATED ENAMEL	pH4	pH5	UNTREATED ENAMEL	pH4	UNTREATED ENAMEL	pH5
1	616	937	764	1,080	1,710	971	1,373
2	257	443	323	540	865	512	612
3	143	253	185	305	516	320	393
4	100	184	129	190	370	297	278
5	70	263	126	198	386	194	257
6	52	147	93	114	260	150	165

*0.1 ppm fluoride in water.

In Table V, the rate of dissolution of a first and sixth layer of enamel before and after removal of the organic constituents is given in terms of weight loss and amounts of dissolved calcium and phosphorus. It will be noted that there is a similar increase in solubility rate from the first to the sixth layers of enamel, irrespective of the presence of the organic constituents. This is evident regardless of whether the solubility rate is measured by weight loss or by the amounts of calcium and phosphorus in solution. It must therefore be concluded that the organic content of the enamel does not measurably contribute to the difference in resistance to acid between the surface and inner enamel. The lower solubility values for the "inorganic" compared to the control enamel may be due to the prolonged heating at 117° C. during the ethylenediamine extraction procedure.

TABLE V
SOLUBILITY RATE OF ENAMEL BEFORE AND AFTER REMOVAL OF ORGANIC CONSTITUENTS*

	CONTROL		ETHYLENEDIAMINE EXTRACTED	
	1ST LAYER	6TH LAYER	1ST LAYER	6TH LAYER
Loss of weight (%)	37.2	45.7	33.9	40.7
Calcium dissolved	6.77	7.93	6.42	7.50
Phosphorus dissolved	3.15	3.75	2.83	3.54

*Percentage loss of weight and milligrams of calcium and phosphorus dissolved from 50 mg. samples of a first and sixth layer of enamel exposed to 5 c.c. of 0.5 M acetate buffer at pH 4 for 1 hour.

DISCUSSION

Our findings support previous observations[1-6] that the surface enamel is more resistant to the action of acid than the inner enamel. The significance of this takes on added weight because our experimental method and sampling procedure differed from those of previous workers.

Within each group it is possible to relate the rate of dissolution in acid to the fluoride content of successive layers of enamel; there was an increase in the solubility rate and a corresponding decrease in the fluoride content from the surface inward. Generally, the greatest increase in solubility rate occurred between the surface layer and the immediate subsurface layer. The decrease in fluoride concentration also was most marked between these 2 layers. Although it is conceivable that other trace elements such as lead, which is present in

greater amounts in surface enamel than in the inner enamel,[15] may also contribute to the difference in solubility rate, our findings show that the organic content of the enamel does not contribute to this effect. Perhaps the organic matrix for intact enamel provides more protection than for powdered enamel, but convincing evidence that this is true has not so far been provided.

The relation between fluoride content and solubility rate is not so consistent when different groups of teeth are compared. It is true that the mottled enamel with the highest fluoride content resisted the action of acid to a greater extent than the enamel from the other groups, while the deciduous enamel with the lowest fluoride content was the least resistent. On the other hand, although the enamel from the under 20 age group from Buffalo has a similar fluoride content to the enamel from the same age group from Chicago, its dissolution rate was significantly lower. It is therefore apparent that these differences in resistance to acid cannot be explained on the basis of fluoride content alone. The possibility that enamel from different geographical areas may differ in composition in regard to other trace elements must also be considered.

When the effect of age on the solubility rate of enamel is examined it becomes increasingly apparent that many factors other than fluoride are involved in the resistance of enamel to acid. Our results show no correlation between solubility rate of enamel and age although there is an increase with age in the fluoride content of enamel. Even with a fluoride concentration of 1,152 ppm, the surface layer of enamel from an over 50 age group dissolved just as rapidly as the surface layer of enamel from the under 20 age group having a fluoride concentration of 499 ppm. Moreover, under the conditions of our experiment, it appears that the subsurface enamel from the over 50 age groups dissolved more rapidly in acid than the subsurface enamel from the under 20 age groups. It is difficult to explain these findings because of the unknown factors involved. The decrease in solubility effected by the accumulation of fluorides and other trace elements might well be masked by other age changes in the enamel which may actually increase its solubility rate. Due to changing dietary conditions it is also possible that teeth formed 50 years ago may differ in structure from those formed in recent years and thus have a different solubility.

Our results show that on exposure to buffer at pH 4 scarcely any fluoride was found in the supernatant even when the concentration of fluoride in the enamel was more than 3,000 ppm, in spite of the considerable weight of enamel dissolved. Under the conditions of our experiment it is possible that (1) little fluoride was dissolved from the enamel or (2) fluoride was readily dissolved but most of it was reprecipitated as calcium fluoride. That fluoride from the enamel can go into solution readily is indicated by the study of Jenkins and Speirs[16] in which as much as 1,800 ppm fluoride was extracted from the enamel surface with acetic acid. It is possible that these workers used larger volumes of acid and so did not exceed the solubility product of calcium fluoride. It is not known to what extent fluoride may be dissolved from the enamel in vivo as a

result of caries. Undoubtedly, reprecipitation of fluoride would be favored by the minute volumes of acid in the proximity of the lesion and the presence of salivary calcium as well as the calcium dissolved from the enamel.

In one series of tests in this study it was observed that the loss of weight in buffer at pH 5 from enamel samples, that had been repeatedly exposed to water and dried at 110° C., was considerably lower than that from corresponding untreated samples. This is interesting in view of the work of Sellman[14] who showed that on heating powdered enamel, above 100° C., its solubility rate in acid was decreased. Evidently, it is important to give uniform treatment to different enamel samples in a comparative study such as this. In this study, efforts were made to meet this requirement and, therefore, the differences reported are believed to be due to different characteristics of the enamel itself and not to extraneous factors.

SUMMARY

1. Solubility in buffers of pH 4.0, 4.5, and 5.0, and fluoride concentrations were determined for successive layers of enamel from deciduous teeth, unerupted permanent teeth, and erupted permanent teeth from persons under 20 years of age, and from persons over 50 years of age. The teeth were obtained from persons who were born and had lived continuously in cities where the water supply contains 0.1, 1.0, or 5.0 ppm fluoride.

2. Solubility increased from the first (outermost) to the sixth (innermost) layer in all teeth at each pH level, and increased most markedly from the first to the second layer.

3. Within each group of teeth the fluoride concentration of successive layers decreased as the solubility of the respective layers increased. However, enamels with comparable concentrations of fluoride showed different solubilities.

4. Mottled enamel, with the highest fluoride concentration, was more resistant to acid than was enamel from the other groups; deciduous enamel with the lowest fluoride concentration was the least resistant.

5. The difference in solubility between surface and subsurface layers was shown not to be related to the organic content of enamel.

6. There appeared to be no relation between age and solubility of enamel in acid.

7. Only small amounts of fluoride were lost from enamel during exposure to acid under the conditions of our experiment.

8. In addition to fluoride concentration, other factors also must affect the resistance of enamel to the decalcifying action of acids. Due consideration must be given to accumulation of trace elements other than fluoride, and other changes which may occur in enamel during its pre- and posteruptive history.

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THE EFFECTS OF FLUORIDE INGESTION ON THE ORGANIC MATRIX OF THE TEETH OF GROWING PIGS

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AN abundant literature has been gathering during the past 30 years on fluorosis, an intoxication affecting mainly the skeletal tissues.[1, 2] This condition is a problem of public health and agriculture. It is a challenge on a theoretical basis since, in spite of the considerable advances already made, the exact mechanism of fluorine poisoning is yet unknown. In previous experiments, the effects of fluoride feeding on Ca^{45} deposition in bones and teeth of pigs have been traced by autoradiography.[3, 4] These studies have revealed a decrease in the growth rate of endochondral bone, dentin, and cementum and also an irregular maturation pattern in these tissues as a result of the high levels of dietary fluorine.

Since the organic matrix could be involved in such changes, similar experiments were initiated using $S^{35}O_4$ as a tracer[5] or histochemical investigations on the demineralized tissues. Also, a new approach, the in vitro uptake of isotopes, has produced interesting results.[6] Microscopic changes observed in the teeth are hereby reported. Similar observations on cartilage and bone are published elsewhere.[7]

MATERIALS AND TECHNICS

In this study, tissues from 12 Hampshire male castrate pigs, 6 weeks of age and averaging 65 pounds were used.[8] The animals, which had been raised on a normal farm ration, were paired and one member of each pair was placed on a diet containing 1,000 ppm sodium fluoride; thereafter the animals were pair fed so as to ensure equal intakes except, of course, for fluoride. After 30 days of feeding, each animal was injected intravenously with 6 mc. of carrier free $S^{35}O_4$. Pairs were sacrificed at 10 minutes, 5 hours, 24 hours, and 10, 30, and 60 days afterward.

The teeth were removed with portions of the jaw. The tissues of the first 3 pairs were quick frozen and then fixed in a mixture of 1 part neutral formaldehyde and 3 parts 95 per cent ethanol. The tissues of the animals sacrificed at 10, 30, 60, and 90 days were fixed immediately. All the teeth were shipped from Oak Ridge to Ottawa in the fixative for further processing and study.

Individual incisors and groups of 2 to 3 molars were demineralized in a mixture of formic acid and sodium citrate at a pH of 4.9[9] and then cut at 10

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microns in low viscosity nitrocellulose by the technic of C. Bélanger.[10] These tissues were stained with hematoxylin and eosin (H and E), hematoxylin-phloxine B and orange (HPO), the Masson trichrome,[11] toluidine blue for metachromasia, the periodic acid-Schiff technic according to Lillie,[12] and the von Kossa silver nitrate reaction for minerals. Some sections were examined unstained under phase contrast. Some sections were incinerated by the technic of Scott.[13] Some of the incisors were embedded in thermosetting polyester resin[10] and ground to a thickness of approximately 20 micron for microradiography by the method of Bohatyrschuk.[14]

OBSERVATIONS

Gross Appearance.—The exposed portion of the teeth of the fluoridized animals did not exhibit any apparent difference in size as compared to the controls. Their general color as well as that of the jawbone was a dull chalky white in the fixative as compared to the opalescent aspect of the controls. After 30 days of fluorine feeding, both incisors and molars showed discoloration and mottling of the enamel in the vicinity of the cemento-enamel junction. This condition was more extensive after 60 days of treatment.

Histology.—In the demineralized sections of teeth, no enamel was preserved. Consequently, the observations were limited to dentin and cementum.

The dentin of the incisors and molars of the control animals was stained red with eosin, phloxine, or the Ponceau-fuchsin of the Masson trichrome. Near the dentino-cementum border, it occasionally appeared paler, with patches of orange-staining material in the HPO or green-staining substance in the Masson trichrome. In some of the incisors, 2 lines stained blue in the HPO were present in the dentin (Fig. 5). These lines ran parallel to the pulp border and were progressively closer to the pulp chamber in the crown portion of the teeth. In the lowermost portion of the root, these lines were wider apart and paler.

Predentin stained pale pink in the H and E preparations, orange in the HPO, and green in the Masson stain. It had uniform thickness and a regular border throughout.

The dentin of the fluoridized teeth has shown wide variations from the above-described pattern. There has been a progressive and patchy loss of acidophilia of the matrix extending from the younger to the more mature portions of the tissue. The tissue was thus stained orange in HPO and green in the Masson trichrome, like the predentin.

At the dentino-predentin junction, an irregular wide band still stained intensely red with the acid dyes (Figs. 1, 2).

In the teeth of the pigs fluoridized for 60 days, there was a sharp limit between modified dentin and what appeared to be still normally staining tissue. The younger root portion was more extensively affected than the older crown portion. The modified dentin, when stained by the Masson technic, has shown patches of light-staining green and pink substance intermixed with unstained areas. With the HPO combination of dyes, the red color of normal dentin had

been completely replaced by a pattern of wavy bands, alternately unstained or pale orange. The outer limit of modified dentin extended to the double basophilic line which appeared unaffected.

In the 60-day animals, all of the dentin appeared modified in the root while, in the crown, a sharp limit was still visible between this type of tissue and an outer portion, much smaller than that of the 30-day teeth, which still appeared normal.

On the other hand, round masses varying in size within narrow limits, appeared in the dentin at some distance from the pulp border (Figs. 1, 5). These masses did not stain much with the above-mentioned dye combinations. At the later stages of intoxication, these globular masses increased rapidly in number but not in size. They were found lined up in wavy bands distributed throughout the dentin which was formed during the period of fluorine feeding.

The dentinal tubules appeared progressively distended, particularly in the younger portion of the root, where they were frequently invaded by elements of the pulp, including blood vessels. In the 30-day animals, where the dentin appeared still strongly acidophilic, a more or less wide portion immediately surrounding the distended tubules stained green with the Masson stain or orange with the HPO.

The predentin in the 30-day animals was very prominent, approximately 3 to 4 times the width of the control, in the root portion. The dentino-predentin junction became irregular. The free surface was also scalloped (Figs. 1, 2, 3) showing, at irregular intervals, wide gaps or breaks marking the openings of distended dentinal tubules into which the elements of the pulp engaged. In the more mature regions of the tooth, the predentin was comparable to that of the controls at this stage.

The changes in the predentin became progressively more extensive in the more advanced cases. In the incisors and molars of the 60-day animal, the whole surface of the predentin was involved. Irregular, localized zones of hyperplasia appeared in the crown which, in 2 recorded instances, were widely penetrated by the pulp (Fig. 2). The tissue surrounding this invasion cavity was not lined by odontoblasts, like the true pulp border, and consequently such irregular masses appeared to grow from the pulp surface only.

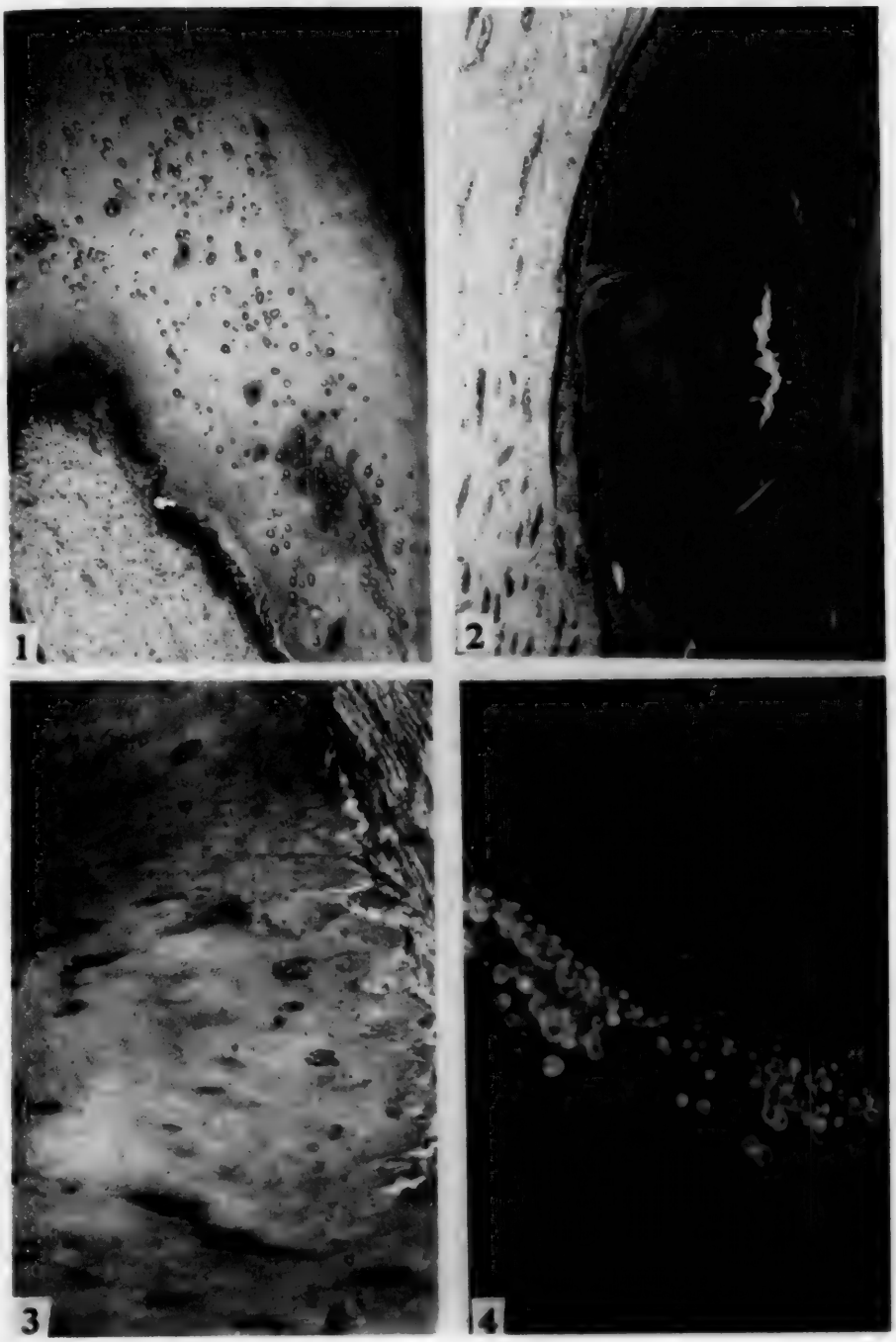
Elsewhere in the crown portion, irregularly formed masses (denticles) appeared in the pulp itself close to the inner wall of the tooth. They were made of concentric layers of organic material, staining like modified dentin and containing rows of shiny globules.

Fig. 1.—The region of root tip of incisor of pig fed fluoride for 30 days. Notice scalloped pulp border, acidophilic band outside predentin, patchy staining of dentin and numerous globular masses (HPO; Orig. mag. ×57).

Fig. 2.—A large, vascularized overgrowth of dentin in incisor of animal treated with fluoride for 60 days. Notice wide acidophilic band next to predentin (HPO; Orig. mag. ×57).

Fig. 3.—The region of root tip of incisor of pig on fluoride for 60 days (PAS stain; Orig. mag. ×57). Notice pale staining dentin with scalloped edges, deep staining material in distended tubules, clusters of globular masses.

Fig. 4.—A portion of dentin of demineralized molar from pig on fluoride for 30 days. Upper half shows regular dentin; lower half shows dentin formed in presence of fluoride. Brilliant globular bodies are in lower half, gathered near border between normal and abnormal tissue (Dark M phase; Orig mag. ×200).



Figs. 1-4.—*For legends see opposite page.*

The pulp cavity of the animals fed sodium fluoride for 30 days appeared lighter stained than that of the controls. This condition seemed to be caused by a decrease in the stainable ground substance rather than a decrease in the cell population. The blood vessels were more numerous and larger. There was a marked increase in the number of odontoblasts present all around the pulp cavity. Where 1 or 2 rows of these cells were normally found in the controls, 4 to 6 rows could be seen in the teeth of the fluoridized animals. Mitotic figures were abundant in the odontoblasts of the bottom rows. In the teeth of the 60-day animals, the odontoblasts were still arranged in 4 to 6 rows in the crown but they decreased progressively toward the root where only 1 row of odontoblasts was seen. These were slender; the cytoplasm was heavily stained; the nuclei were small and dark.

The cementum was observed in similar fashion. In the normal incisors and molars of the pig, young cementum, or precementum, at the outer border of the root stained like predentin. The mature tissue was practically homogeneous and acidophilic apart from small halos around the young cementocytes which stained like precementum, orange with HPO, or green with the Masson trichrome.

In the teeth of the animals fluoridized for 30 days, the first change observed has been irregular zones of unstained tissue within the mature cementum, close to the dentino-cementum junction. In these areas, appeared globular masses similar to those described in the dentin. They increased progressively in number but never to be as numerous as in the adjacent dentin. At 60 days, the outer band of precementum had become wider and its true limits were difficult to appreciate. The cementum had shown a progressive decrease in acidophilia so that a basic architecture, similar to that of a tendon, became evident. This pattern was in form of a succession of acidophilic bands disposed perpendicularly to the surface of the tooth and separated by rows of cells embedded in an orange- or green-staining matrix. There was evidence of cell degeneration and decreased formation of new cementum.

Histochemistry.—

1. *Metachromasia:* Metachromatic staining of normal teeth with toluidine blue has revealed, in previous work, a weak reaction of predentin followed by a strong reaction at the dentino-predentin border in mineralized sections. In demineralized sections, the whole dentin is metachromatic.[15, 16] Demineralized sections of the incisor teeth, in the present series, were stained with 0.5 per cent toluidine blue and examined in mineral oil in order to preserve the reaction.[17] The controls have shown a weak stain over predentin and a stronger even stain of the mature dentin. The 2 parallel lines which took hematoxylin electively were now considerably more metachromatic than the adjacent dentin when stained with toluidine blue.

The dentin formed during fluorine feeding, on the contrary, did not stain metachromatically with toluidine blue and appeared as pale blue. As the amount of newly formed dentin increased in the 60-day pigs, there was a progressively larger portion of the tooth which was not metachromatic. The

modified dentin did not extend beyond the growth lines; these lines remained well defined and characteristically metachromatic throughout the experiment.

The ground substance of the pulp was moderately metachromatic in the controls while progressively paler in the fluoridized animals.

2. *Periodic acid-Schiff reaction:* In the demineralized teeth of the control animals, the PAS staining material was distributed as follows: the pulp was weakly and diffusedly stained, predentin appeared practically negative, dentin was generally and moderately stained. In the molars, an occasional lamellar arrangement of weak and moderate staining substance was observed. At the dentino-predentin junction, there was a sharp line of more intensely staining substance. The dentino-cementum junction was also marked by a strong reaction. The cementum showed a moderately stained inner region and a weakly stained outer portion, occasionally marked by more intensely stained striations corresponding apparently to the arrangement of fibers previously described.

The fluoridized teeth, particularly the molars, showed a patchy loss of PAS staining substance. Above the zone of initial formation of the clear globular masses, the dentinal tubules were often distended and contained intensely staining material (Fig. 3). The proximal pulp of the 60-day animal was also very intensely stained. The reaction appeared to be localized to the cytoplasma of the stellate cells. The odontoblasts were always unstained or weakly positive.

The organic matrix of fluoridized *cementum* has also shown a progressive decrease of the PAS reaction. The cementoblasts were always negative or weakly positive. The cementocytes appeared moderately positive in the normal tissue and in the tissues of the treated pigs.

3. *Silver nitrate:* All the tissues referred here as demineralized were negative when submitted to the von Kossa test for lime salts with a maximal exposure of 60 minutes to a 1.5 per cent solution of silver nitrate.

4. *Phase contrast:* Unstained sections of the bones and teeth were coverslipped with Permount and examined by phase contrast microscopy. The Dark M objectives (A.O.) produced images with the most contrast described below. All parts of the normal teeth appeared either black or in various shades of gray. The globular masses described previously, appeared as very bright bodies against the dark background (Fig. 4). Their outer border was well defined and brightest. The masses were, at first, dispersed and at some distance from the pulp border at a point which appears to be the onset of formation of modified dentin (Fig. 4).

In the teeth of the 30-day animals, a line made of smaller, also brilliant particles appeared at the junction of predentin and dentin. Both features were more extensive in the root portion of the tooth. At 60 days, this latter aspect had developed considerably and seemed to involve the dentinal tubules. The highly modified dentin of the 60-day animals showed few globular bodies but irregular stratified layers of small bright particles, separated by layers of more homogenous, darker substance.

In *cementum,* only the bright globular masses were observed. These were dispersed throughout the tissue but were apparently more numerous in the more mature portions.

5. *Spodography:* The incinerated preparations were examined by dark field microscopy. The demineralized sections of teeth of the control animals were not expected, of course, to yield abundant ashes. In normal tissues, the contour of the blood vessels was marked by a light deposit. The border of the pulp cavity in the teeth was marked by the presence of a regular fine striation, representing apparently the intercellular substance between the odontoblasts (Fig. 6). The substance of predentin and dentin was generally negative (Fig. 6). A surprising feature of dentin was the appearance of the growth lines which produced a relatively high ash content (Fig. 6). The dentin of molars has occasionally shown a series of fine parallel bands separated by bands of low ash content.

The teeth of the pigs which were fed sodium fluoride for 30 days revealed an ash deposit apparently in the shape and location of the globular masses isolated or conglomerated. The tissues of the 60-day animals produced a considerable and increasing amount of ash in the form of a fine diffuse deposit over the newly formed dentin (Fig. 8) but not over predentin.

The effect of ammonium acetate: It is possible to consider the ash content of the fluoridized teeth as representing original deposits of calcium fluoride. This was previously reported as a probable occurrence in fluorosis.[18] Calcium fluoride is known to be only slightly soluble in acids used in demineralization. On the other hand, this substance is soluble in solutions of ammonium salts.[19]

Sections of teeth of fluoridized animals were placed in a 2 per cent aqueous solution of ammonium acetate for 24 hours. They were then incinerated or mounted unstained for phase contrast microscopy. After this treatment, the majority of the globular masses have disappeared from the cementum and dentin; some were actually found partly dissolved. On the other hand, the substance which yielded the diffuse, fine ash did not seem to be affected by the ammonium acetate, as evidenced by the spodogram.

6. *Microroentgenography:* In order to observe the densitometric pattern of the mineralized tissue, an incisor of the pig fluoridized for 60 days was embedded in thermosetting polyester resin, ground to a thickness of approximately 20 microns, and mounted on thin foil for microroentgenography. This

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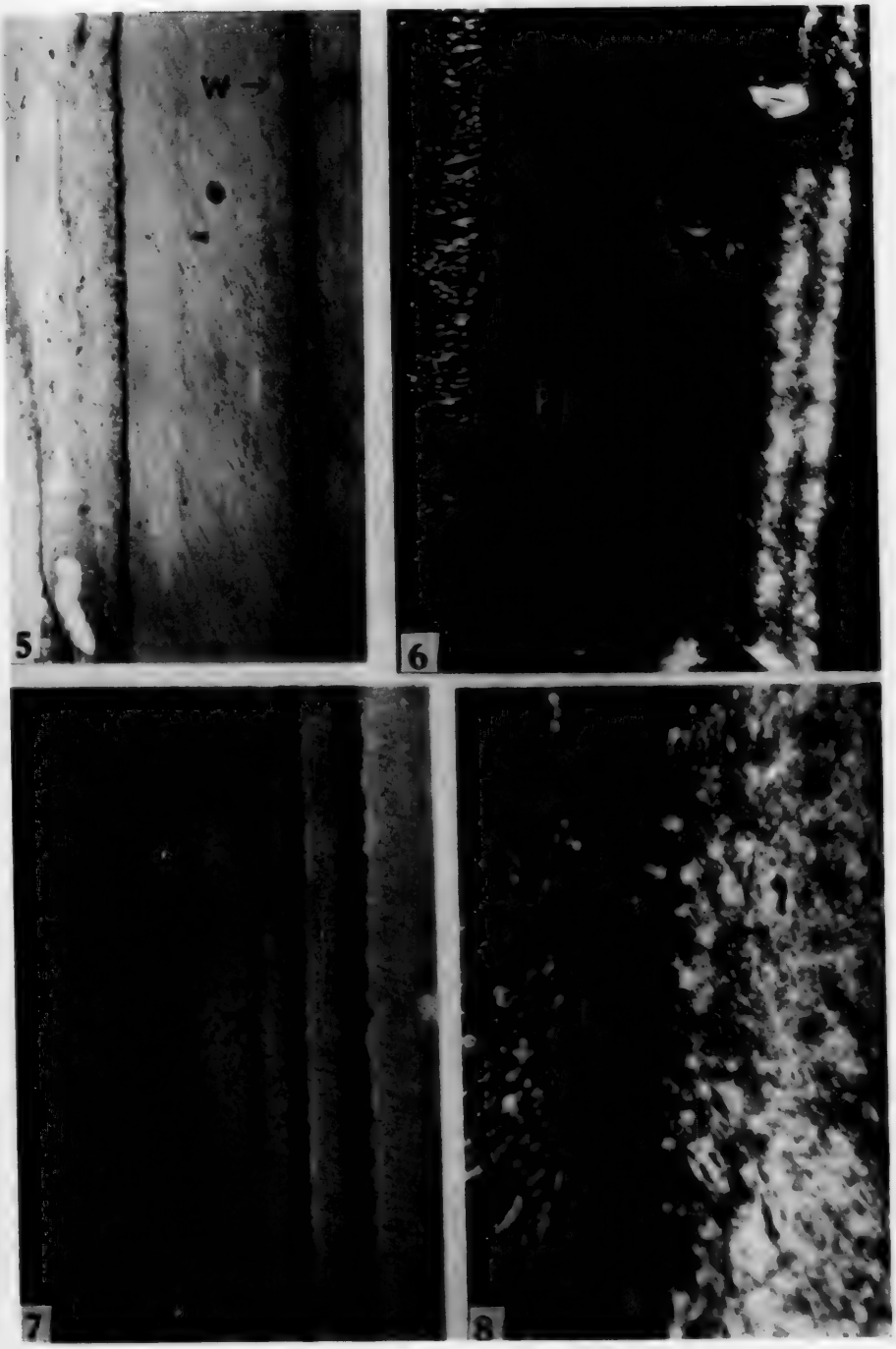
Fig. 5.—Upper portion of incisor after 30 days on fluoride diet. Notice from left to right, dilated vessels of pulp, globular masses, weaning line (*W*), neonatal line (*NN*) (HPO; Orig. mag. ×32).

Fig. 6.—Spodogram of normal incisor of pig. From left to right, proximal pulp shows fine strips of ash between odontoblasts; weaning line and neonatal line show an abundant fine deposit of ash. *PD*—predentin. (Orig. mag. ×200.)

Fig. 7.—Microroentgenograph of mineralized dentin of incisor from pig on fluoride for 60 days. On right, regular normal prebirth dentin is interrupted by dentinal tubules. Neonatal line and weaning line are conspicuously transparent. In region located inside weaning line (on left) minerals are in globular form. Mineral units are further apart from one another in more recent portion of tissues (extreme left). (Orig. mag. ×200.)

Fig. 8.—Spodogram of incisor of pig on fluoride for 60 days (Orig mag. ×200). Notice abundant, homogeneous ash deposit in proximal dentin, enlarged ash free predentin (*PD*).



Figs. 5-8.—*For legends see opposite page.*

method has revealed, in the portion of the tooth formed previous to fluorine feeding, an even density interrupted only by the parallel dentinal tubules (Fig. 7). The neonatal and weaning lines were highly permeable to the radiations, indicating that the spodogram of the demineralized sections (Fig. 6) is a precision record showing what really must be a small amount of mineral yielding substance as compared to normally and even abnormally mineralized dentin. Under these two lines, the dentin formed during fluorine feeding consisted of apparently uniform round masses (Fig. 7); these were more closely agglomerated in the outer portion of the modified tissue.

DISCUSSION

The development of dentin is a biphasic phenomenon consisting of apposition or accretion and consolidation or maturation. These two stages in dentin formation are dependent on a regulated organic and mineral metabolism controlled by general and local factors. The present series of experiments cast some light on the behavior of the mucoid fraction of the ground substance in development, under normal conditions and after chronic fluoride ingestion.

This mucoid fraction, which presumably consists of mucopolysaccharides in part sulfated,[20] appears first in predentin. It is generally considered to be a product of secretion of the odontoblasts. Autoradiographic observations with $S^{35}O_4$[20] and C^{14} glucose[21] have revealed uptake of the radioelements by predentin and transit of such substances into dentin. In the present series of observations,[5] this transit in the normal teeth was in the form of a diffuse penetration of dentin followed by a fairly rapid disappearance from the tissue. At the dentino-predentin junction, there is a suggestion that a larger concentration of the mucoid fraction might develop just prior to mineralization.[16] When temporary mineral deficiency appears, such as at time of birth and weaning, a portion of the constantly growing tissue might not receive full mineral implement and, later, be recognizable as a differently stained band in sections of the organic dentin (Fig. 5) and also by x-ray patency in a microradiograph (Fig. 7). These are the neonatal[22] and weaning lines.

Throughout normal dentin, the concentration of polysaccharides is greater than that of predentin as estimated by the intensity of the PAS, and metachromasia.[16] The present observations have confirmed this fact. It is likely that the polysaccharides are in combination with a protein which would be responsible for the intense acidophilia generally observed in demineralized dentin.

In the present material, an unexpected concentration of ash (Fig. 6) has been observed over the neonatal and weaning lines in demineralized sections. This phenomenon will be discussed along with the effects of fluoride.

Staining differences between normal cementum and precementum have been found comparable to those of dentin and predentin.

The demineralized incisors and molars of fluoridized animals have revealed staining properties related to an absence of maturation of dentin and reactive overproduction of predentin.

The increased radiosulfate uptake by predentin, reported previously,[5] ascribes in part the irregular predentin hyperplasia described by Irving[24] to an increased synthesis of sulfated mucopolysaccharides. However, after 2 months of fluoride ingestion, it seems that the predentin reaction has ceased and that the dentin has lost a large amount of its stainable mucopolysaccharides.

The presence of large bright spherules (Figs. 1, 3, 4) has been recognized for a long time in bone[25] and dentin[18] and theoretically identified as calcium fluoride. The present observations related to solubility of these masses in ammonium acetate are in favor of this opinion.

On the other hand, the spodograms of demineralized teeth have revealed an abundant white ash over the portion of dentin (Fig. 8) deposited during fluoride feeding. Incinerated cementum, bone, and cartilage[7] revealed progressive deposits of apparently similar material in animals fluoridized for 30 and 60 days. In normal animals, minor, well-localized deposits of blue-white ash were observed over the demineralized cartilage of the epiphysis of long bones[7] and, curiously, over the neonatal and weaning lines of dentin (Fig. 6). Such deposits were still visible after treatment with ammonium acetate which dissolved the large, shiny globules and produced negative images of them in the spodograms.

What is this substance which is represented in the spodograms by the fine blue-white ash? Recent studies with the help of Ca^{45} have revealed that cartilage contains a cation binder capable of fixing calcium in vitro[23] and in vivo.[26] Experiments with ion exchangers by Neuman, Boyd, and Feldman[27] indicate that this substance might well be chondroitin sulfate. This material seemed to accumulate in the rib cartilage of rachitic children[23]; it appeared to decrease in aging trachea, in places where this structure became mineralized.[23]

Autoradiographs of in vitro uptake of Ca^{45} have revealed an exaggerated concentration of this isotope over the accretion sites of dentin, cementum, and bone.[6] In normal and fluoridized animals, there was also an uptake of radiocalcium over the neonatal and weaning lines.

Amprino[28] and Vincent[29] have both demonstrated for bone an early sulfated phase during which accretion and dissolution may take place easily. The observations with Ca^{45} uptake indicate that a cation binder, presumably chondroitin sulfate, plays a major role in the site of mineral accretion in bones and teeth, presumably by concentrating calcium previous to its release and combination with phosphate. The fine blue-white ash deposits in dentin, cementum, and bone in fluoridized animals can be interpreted as an accumulation of an organic calcium precursor.

In conclusion, after prolonged ingestion of a relatively large daily dose of fluoride, deformed immature and abnormally mineralized teeth are observed in the pig. The changes affect not only enamel but also dentin and cementum as well as bone.[1, 2, 7] The faster growing incisors show more extensive changes than the molars.

SUMMARY

Progressive changes have been recognized in the dentin and cementum of the teeth of pigs fed a diet containing a large dose of fluoride (1,000 ppm) for periods of 30 and 60 days.

The changes were more extensive in the incisors than in the molars. Dentin and cementum have shown a graded decrease of staining by the periodic acid-Schiff technic and toluidine blue metachromasia.

Predentin and precementum were hyperplastic, occasionally tumoral. The number of odontoblasts and cementoblasts was increased at 30 and decreased at 60 days.

The pulp cavity showed hypervascularization and decrease in stainable ground substance.

Round, clear masses, probably containing CaF_2, appeared in newly-formed dentin and cementum of fluoridized animals.

Homogeneous, compact, blue-white ash of unknown identity was detected in spodograms of demineralized sections of dentin and cementum from fluoridized animals.

The significance of these findings in relation with normal mineralization is discussed.

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METABOLIC SIGNIFICANCE OF COLLAGEN IN TOOTH STRUCTURE

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DENTIN has been classified as a collagen and enamel protein as a keratin structure.[1, 2] It is the purpose of this report to evaluate the results of recent studies of dentin and enamel proteins[3, 4] and compare them to existing knowledge of protein classification.

COLLAGEN IDENTIFICATION AND CHARACTERISTICS

Collagen, an albuminoid protein found in ligaments, tendons, and bones throughout the human body,[5] constitutes 30 per cent of total body protein and contains over 70 per cent of the body's store of glycine, proline, and hydroxyproline.[6] The partial hydrolysis of steer-hide collagen produces a high yield of the tripeptide, glycyl-prolyl-hydroxyproline.[7] The molecule is fibrous, rather than globular, and consists of 3 coaxial helical polypeptide chains with 30 amino acids per turn and about 7 turns in each repeating unit which is 618Å long; the hexagonal stacking is stabilized by cross linkages through hydroxyproline side groups.[8]

Gustavson[9] stated that the hydroxyproline residue of collagen plays the same governing role for the stability of collagen as the cystine residue does for keratin. Ward and Lundgren[10] have noted that keratins have a typically high cystine content and proline-to-cystine bonds are common; the keratinizing process is the oxidation of sulfhydryl groups (—SH) to the disulfide linkages (—S—S) between protein chains with the resulting insolubility of keratins.

Collagen has been stated as controlling the calcification of bone and crystallization of apatite in bones.[11] The 218Å and 650Å repeating units of collagen are parallel to and coincide with the repeating structure of apatite upon which the collagen is layered.

A white fibrous precipitate occurs upon mixing collagen with chondroitin sulfuric acid (galactosamine sulfate analog of hyaluronic acid at pH values from 2 to 8 or in 0.03M $CaCl_2$ solutions).[12-15] Thus, by increasing calcium concentration, an optimum concentration of calcified precipitate is formed with staining characteristics of polysaccharides. Beyond this point, additional calcium produces a more calcified precipitate which becomes deficient in polysaccharide. If denatured collagen is used, calcification of the precipitate is eliminated but polysaccharide staining is retained.

The opinions or assertions contained in this article are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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PROTEINS OF TOOTH STRUCTURE

Teeth represent complex calcified structures in which calcification is differentiated in 3 distinct anatomic regions: enamel, dentin, and cementum. The protein matrix of dry enamel amounts to about 1 per cent (0.49 to 1.95 per cent of the tissue).[16] Dry dentin contains on the average 22 per cent of protein matrix.[17]

Within the last few years there have been exhaustive amino acid analyses of the proteins in teeth. Two groups[3, 4] presented similar findings with only minor differences.

In order that the values of amino acids presented may be used for identifying the types of protein, all of the results have been converted from percentage of amino acid in 100 Gm. of protein to ratios of amino acid molecules present, equating the lowest ratio of amino acid to a value of one. These ratios were compared to similar ratios computed for collagen[5] and a number of keratins: (a) soft keratin (skin), (b) intermediate keratin (feathers, wool, hair), and (c) hard keratin (horn).[4]

Collagen and the keratins were noted to have an average ratio of 15 for the glutamic acid values. Therefore, the tooth protein analyses were further adjusted so that the number of glutamic acid molecules in each case was equal to 15 for comparative purposes. This summary is presented in Table I.

TABLE I

COMPARISON OF AMINO ACID COMPOSITION OF PROTEIN OF ENAMEL AND DENTIN TO AMINO ACID COMPOSITION OF COLLAGEN AND KERATIN

	COMPOSITION							
				KERATIN				
AMINO ACIDS	COLLAGEN	ENAMEL*	DENTIN*	SKIN	HAIR	WOOL	FEATHER	HORN
Tryptophane	0	(.1)	0	1	1	1	1	1
Cystine	0	1	2	1	10	8	8	10
Tyrosine	1	2	1	3	2	5	3	5
Methionine	1	2	1	2	1	1	1	2
Histidine	1	2	1	1	1	1	1	1
Lysine	6	6	5	4	3	3	3	4
Arginine	10	9	9	6	8	9	9	9
Phenylalanine	5	9	2	1	3	3	7	4
Isoleucine	8	3	2	4	5	5	10	5
Leucine		8	6	5	8	9	13	10
Valine	5	7	5	5	7	7	17	7
Threonine	4	9	5	2	9	8	9	8
Serine	6	16	8	14	13	13	29	10
Aspartic Acid	9	9	9	5	8	8	12	9
Glutamic Acid	15	15	15	9	13	15	15	14
Proline	24	11	29	2	11	10	19	11
Hydroxyproline	20	11	21	0	0	0	0	0
Glycine	58	55	62	16	4	7	12	11
Alanine	19	21	20		3	6	11	3
Hydroxylysine			2					
Totals	192	196.1	205	81	110	119	189	124

Sources: Compiled from data in Battistone, G. C., and Burnett, G. W., J. D. Res. 35: 255-272, 1956; Hess, W. C., and Lee, C., J. D. Res. 33:62-64, 1954; and Bowes, J. H., and Kenten, R. H., Biochem. J. 43:358, 1948.

*Ratios to 15 parts glutamic acid.

COMPARISON OF PROTEIN IN TOOTH STRUCTURE TO COLLAGEN

Inasmuch as the identifying features of collagen are low cystine but high hydroxyproline and glycine, and the identifying features of keratin are high cystine but no hydroxyproline, it is a relatively simple matter to classify the protein of enamel. The collagen molecule derives its stability from hydroxyproline bonding between the protein chains while keratin derives its stability from the cystine (S—S group) bonding between protein chains. In these essential features, the protein of enamel corresponds closer to that of the collagen molecule than the keratin molecule.

On the other hand, it will be noted that there are several general differences between the proteins of known keratins and the proteins of enamel. (a) Enamel protein has a negligible amount of tryptophane as compared to an amount 10 times greater in keratin, (b) more than 10 times as much cystine is found in keratin as in enamel protein, (c) no hydroxyproline has been found in any known keratin while enamel protein has a prominent amount, (d) glycine is present in enamel protein in an amount 8 times greater on the average than any known keratins, and (e) alanine is present in enamel protein in an amount 3 to 4 times greater than any known keratin.

Sullivan[18] identified a calcium mucoproteinate in the prism sheath areas of enamel. Since the mucopolysaccharide of this complex has been identified as chondroitin sulfate[19] and the calcification process involves calcium, chondroitin sulfate, and collagen, it is suggested that Sullivan's "calcium-mucoproteinate" is a complex of calcium-chondroitin sulfate collagen.

Since teeth are the most highly calcified tissues of the body, it would be logical that they be dependent on the influence of collagen in their formation.

The isolation of pure proteins from enamel may be necessary before these materials can be exhaustively characterized.

SUMMARY

The amino acid patterns of tooth structure, known keratins, and collagen have been compared and the proteins of enamel pointed out to resemble collagen more closely than keratin.

The author acknowledges the aid of Gordon H. Rovelstad, CDR (DC) USN, in the preparation of this manuscript.

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THE EFFECTS OF LOCAL INJECTION OF SOMATOTROPHIC HORMONE IN SMALL DOSES INTO THE ORAL MUCOSA OF YOUNG ADULT RATS

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THE stimulating effects of somatotrophic hormone on general and specific organ growth and weight retention has been widely studied in both intact and hypophysectomized animals.[1, 2, 3] This growth stimulation is thought to be the result of increased protein retention and anabolism.[4, 5, 6] The general anabolic effect of the hormone may be altered by dietary deprivation, especially restrictions in dietary proteins, and may then act only on selective tissues such as bone and cartilage.[8]

The local stimulating effects of somatotrophic hormone on connective tissue have been described as an increase in fibroblasts and collagen, as well as an increase in granulation tissue around areas of inflammation.[9, 10, 11] Increased thickness of the skin with increase in the size of hair follicles has also been reported following local injection of growth hormone.[12]

The present study was undertaken in order to observe the effects of local injection of small doses of growth hormone on the oral mucosa of young adult male rats both in vivo and in vitro.

MATERIAL AND METHOD

Twenty-five Long-Evans strain male rats, weighing an average of 240 Gm. at the beginning of the experiment were divided into 2 groups. Group 1 (15 animals) received injections of 0.5 mg. of STH* in 0.1 c.c. of distilled water, buffered to a pH of about 10.0, 3 times weekly into the mucobuccal fold below the maxillary left first molar. The control group received similar injections of distilled water, buffered to a pH of 10.0. The experimental period lasted 6 weeks, with food and water being provided ad libitum.

At the end of the experimental period, the injection site was dissected out and divided into two specimens, one of which was used for tissue culture study, while the other was prepared for routine histologic examination. The

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*STH used was beef STH, Armour Lot No. 369-266 which contained TSH contaminant. The authors wish to thank Dr. S. L. Steelman, Head, Biochemical Research Department, the Armour Laboratories, for the generous supply of STH.

maxilla, femur, and selected organs were also dissected out for histologic study. All histologic specimens were fixed in formalin, cut at 7 microns, and stained with hematoxylin and eosin.

The oral mucosa to be used in in vitro studies was cultured in Carrel flasks for 2 additional weeks, without further treatment, for live cytologic observations. The tissues were maintained in a chick embryo extract-plasma coagulum, some with added nutrient of Hanks balanced saline, embryo extract, and horse serum ultrafiltrate. The nutrient fluid in the latter cultures was changed at 3-day intervals.

Tissues were cut into 1 by 1 and 1 by 2 mm. strips, consisting of epidermis, dermis and, in some cases, underlying muscle. The strips were oriented in the Carrel flasks so that the 2 or 3 layers lay in a single plane, parallel to and against the lower surface of the flask. Temperatures were maintained at 37° C. and pH at about 7.2.

OBSERVATIONS

Histologic observation of the oral mucosa treated with growth hormone showed as its most striking histologic change, a marked epithelial enlargement with rete peg proliferation and an increase in the subepithelial connective tissue. This epithelial enlargement was noted in all animals and appeared to be much more striking than that observed following the local irritation due to the injection of the control solution (Figs. 1, 2, 3, and 4).

At the site of experimental injection, there was an increase in the thickness of the keratin layer and, in many instances, epithelial cell whorls were noted within this layer. The stratum intermedium was also markedly widened (Fig. 5). The individual cells were increased in size. Many had two nuclei which stained distinctly and appeared to be joined together (Fig. 6). Multiple nucleoli were observed within these nuclei. The germinal epithelial layer showed some evidence of mitosis, but the number of mitotic figures seen in this group was small. In some areas, heaping of epithelial cells at the basement membrane extending into the subepithelial connective tissue was also noted (Fig. 7).

The corium showed an increase in the number of fibroblasts, and collagen fiber bundles appeared in great abundance.

Inflammatory infiltrate, consisting primarily of lymphocytes and plasma cells, was more evident in the control group than in the experimental animals.

TISSUE CULTURE

Cultures of the adult oral mucosa injected with STH showed epithelial cells migrating as early as 18 hours after explantation and fibroblasts later, a reversal of the usual chronological order of appearance of these cells. At 40 hours, the epithelial sheets tended to resemble cultures of fetal more than adult tissues in extent and regularity of outgrowth. Fibroblasts seemed relatively inhibited for the first 4 or 5 days; these were fewer and exhibited less migratory activity than normal (Fig. 8).

The epithelial cells were larger, more vacuolated, and more granular than in the control cultures. Most had 2 nuclei; others had 3 or more. The nuclei

Fig. 1.

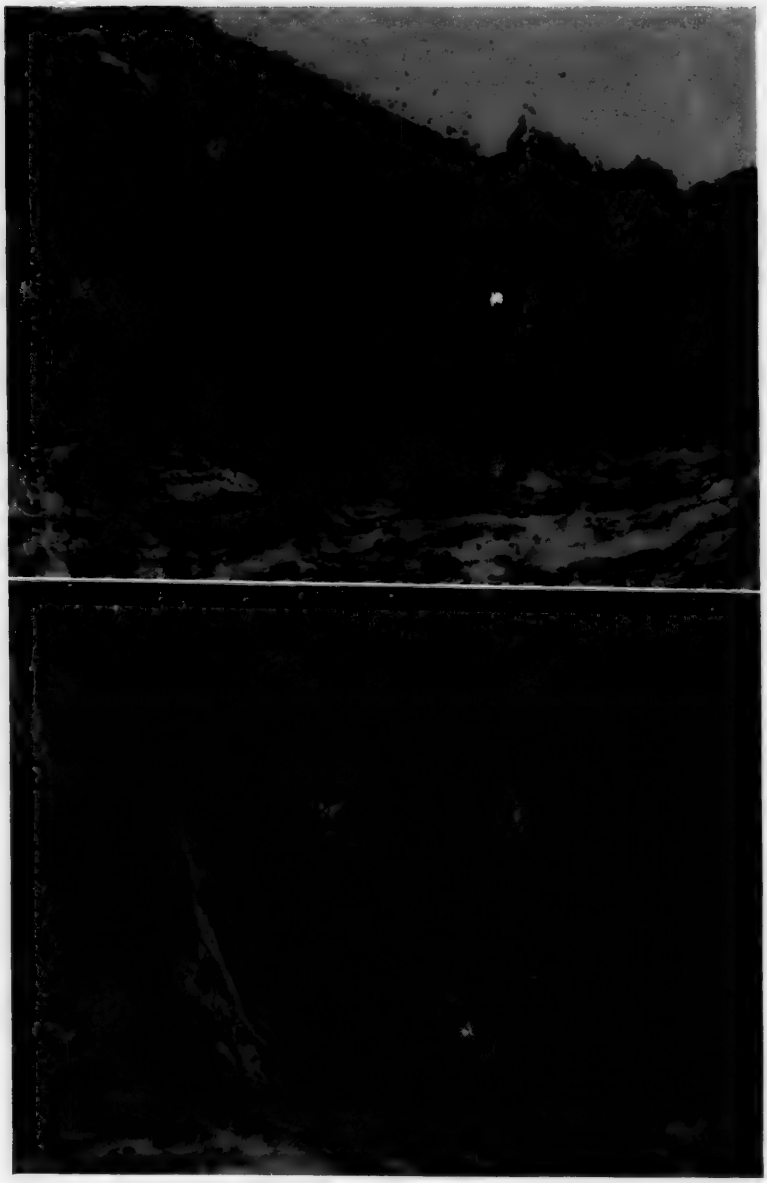

Fig. 2.

Fig. 1.—Section of rat oral mucosa treated with local injections of somatotrophic hormone. Note acanthosis of epithelial layer. (Hematoxylin-eosin; orig. mag. ×120.)

Fig. 2.—Higher magnification of epithelium shown in Fig. 1. Note number of binucleate cells. (Hematoxylin-eosin; orig. mag. ×310.)

Fig. 3.

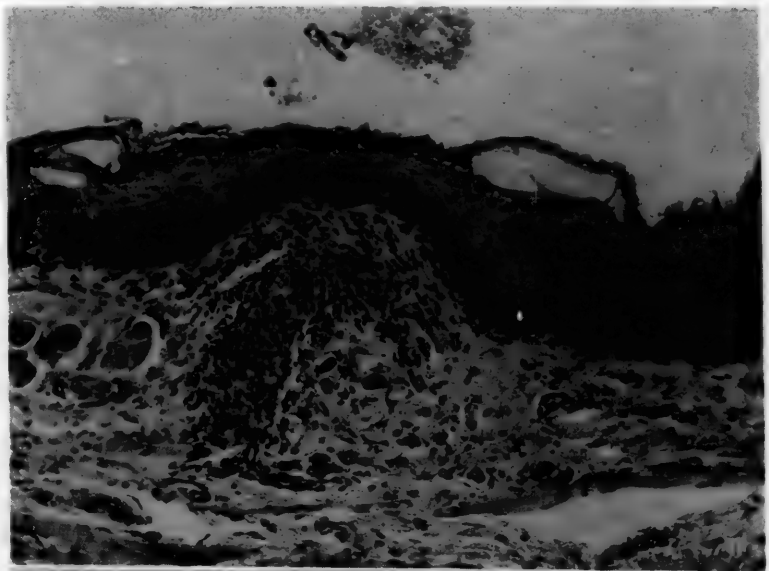

Fig. 4.

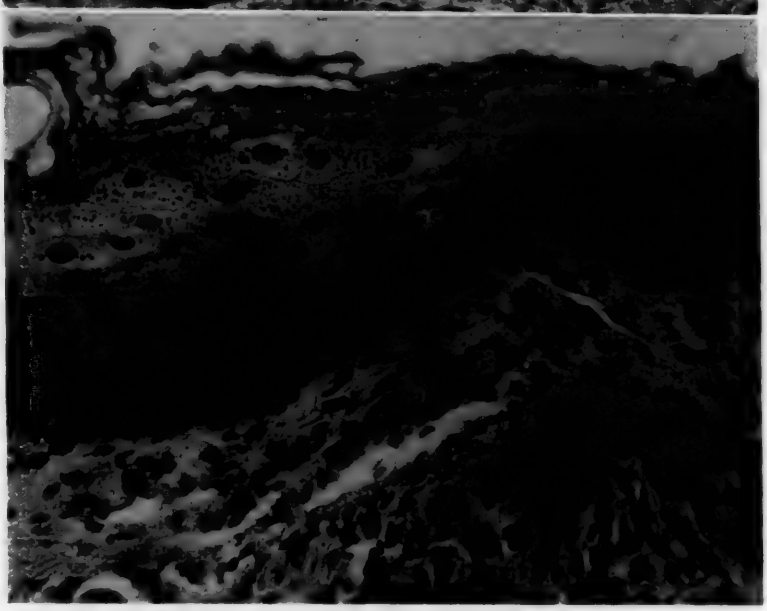

Fig. 3.—Section of rat oral mucosa treated with local injections of the control solution. Note inflammatory infiltrate. (Hematoxylin-eosin; orig. mag. ×120.)

Fig. 4.—Higher magnification of epithelium shown in Fig. 3. Note regular appearance of epithelial nuclei. (Hematoxylin-eosin; orig. mag. ×400.)

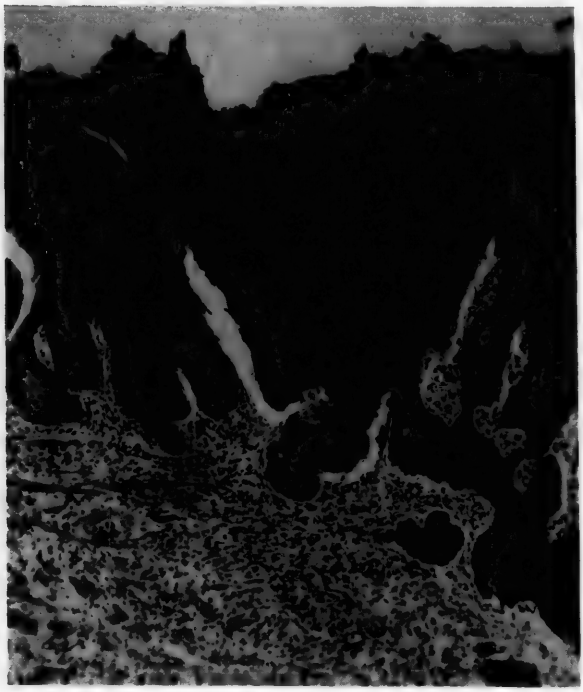

Fig. 5.—Section of rat oral mucosa treated with local injections of somatotrophic hormone. Note thickness of keratin layer and proliferating rete pegs. (Hematoxylin-eosin; orig. mag. ×120.)

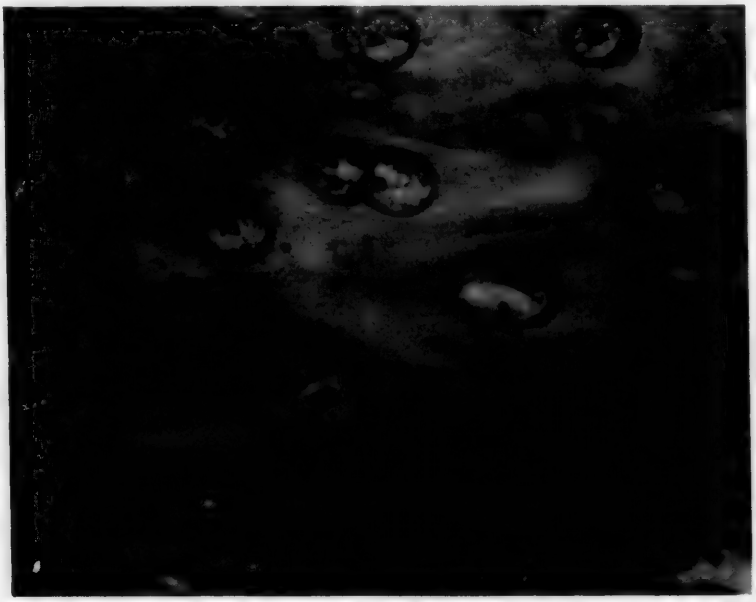

Fig. 6.—Area of rat oral mucosa treated with local injections of somatotrophic hormone. Note binucleate cells. (Hematoxylin-eosin; orig. mag. ×990.)

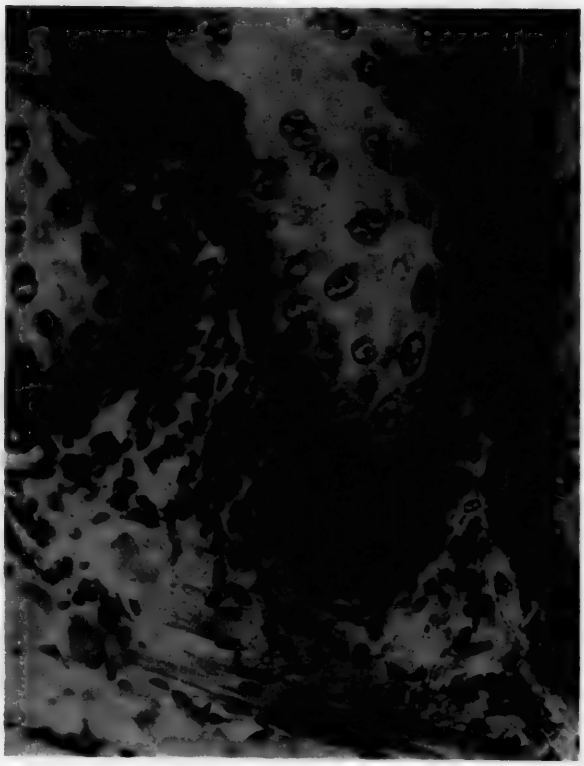

Fig. 7.—Area of rat oral mucosa treated with local injections of somatotrophic hormone. Note binucleate cells as well as large number of nuclei in basal layer. (Hematoxylin-eosin; orig. mag. ×220.)

Fig. 8.—Living, unstained section of adult rat oral mucosa treated in vivo with local injections of somatotrophic hormone and growth in tissue culture for 36 hours. Epithelium and connective tissue are present in the original explant (dark), but few fibroblasts are seen at this time in outgrowth. (Orig. mag. ×50.)

of the double-nucleated cells generally remained attached to each other even during migratory activity, apparently separated only by adhering nuclear membranes. Fibroblasts were not noticeably changed.

At 4 to 5 days in vitro, epithelial sheet migration slowed down considerably and fibroblastic activity and numbers increased. This difference was usually augmented with continued maintenance of the cultures during the following week.

COMMENT

The presence of binucleate cells in oral tissue has been reported by Ludford,[13] who stated, "At the commencement of keratinization there is usually to be distinguished an increase in the volume of cytoplasm in proportion to the nucleus. Such a disturbance of the nuclear cytoplasmic relationship leads to attempts on the part of the nucleus at readjustment and there follows amitotic division. This is of common occurrence in the cells near the centers of keratinization in tar tumors and results in the formation of binucleate cells. . . . What seems to be amitosis occurs in the epidermis of the rat's tongue." Occasional binucleate cells in the gingivae of monkeys treated with estrin and gonadotropic hormone have also been observed.[14]

The formation of binucleate cells has also been observed in human and animal tumors[15, 16] and in the regenerating liver cells following repeated liver injury by injection of carbon tetrachloride.[17] A mechanism for this change in nuclear population following liver injury has been suggested by Hoffman, Himes, Klein, and Poulas[17] who noted, "After injury and stimulus to regeneration, some of the surviving cells double their nuclear DNA in preparation for cell division of this group, many divide and revert to their original class. However, some remain undivided. In this manner the nuclear population changes. . . . It is possible that with repeated stimuli to regeneration, whether induced by tissue injury or by humoral or other mechanisms, there may occur a gradual transformation of nuclear types along with associated cellular metabolic changes, which eventuate in a cellular population with altered growth potential."

Increasing binucleation has also been observed in the transformation of normal to precancerous to cancerous cells in animal tumors.[16]

In our material, we have noted the presence of binucleate cells in oral mucosa sections of the control animals. A marked increase in binucleate cells as well as an over-all increase in the size of the individual cell and the whole epithelial layer, however, appeared to be a constant, direct effect following local injection with somatotrophic hormone. In this connection, a recent study of the effects of hypophysectomy on the mitotic activity of oral tissues showed a decrease in mitosis in oral epithelium and connective tissue to follow removal of the pituitary in rats.[18]

SUMMARY AND CONCLUSIONS

Twenty-five Long-Evans strain male rats weighing an average of 240 Gm. at the beginning of the experiment were divided into 2 groups. Animals in

Group 1 received intraoral injections of somatotrophic hormone in small doses 3 times weekly for a total of 6 weeks. Animals in Group 2 received similar injections of a control solution.

It was observed that injections of somatotrophic hormone led to a marked acanthosis and increased keratinization of the epithelium, a striking increase in binucleate cells in the epithelium, and an increase in connective tissue fibers in the corium of the experimental site.

Tissue culture of the experimental site showed migration of the epithelial cells prior to migration of fibroblasts after explantation, the epithelial cells appeared larger, more vacuolated, and more granular than those seen in the control cultures, and most epithelial cells from the experimental site had 2 or more nuclei.

The authors wish to acknowledge the technical assistance of Marcella Hazan, Dr. Biol., in the preparation of the study.

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THE PRESENCE OF BACTERIA IN THE HEALTHY GINGIVAL SULCUS

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WAERHAUG and Steen have concluded that the normal gingival sulcus is sterile, being subject to bacterial invasion only after subgingival calculus formation.[1] They sterilized the teeth and gingivae of dogs and human beings by applying equal parts of a 5 per cent iodine solution and glycerin for 3 minutes, and then dried them with blasts of air. Thin steel blades were inserted into the sulcus, removed, and dropped into culture media. When pure cultures of bacteria were introduced into the normal sulci of men and dogs, the microorganisms disappeared within 48 hours. They suggested that a bacteriologic examination will indicate whether or not calculus has been completely removed from a periodontal pocket.

The authors have attempted to confirm the observations of these investigators. If their findings can be corroborated, we must revise our theories of calculus formation and certain ideas of the nature of periodontal disease.

PROCEDURE

For the purposes of this study, the gingival sulcus is defined as the area between the tooth and the free gingiva extending from the gingival margin to the most coronal portion of the epithelial attachment.

Material was obtained from dental students, from adult patients who had completed treatment in the periodontia clinic, from children under 12 years of age from the pedodontic clinic and from certain wild and domestic mammals. Clinically healthy mouths were selected and sulci were chosen which seemed to be free from deposits when examined under a magnifying lens.

The quadrant was isolated with cotton rolls and saliva ejector, then dried with sterile gauze sponges. The sponges were used as a blotter. Any wiping was from gingival margin to teeth to avoid forcing material into the sulcus. A fine hooked explorer (No. 17, S.S.W.), with the point blunted to prevent tissue damage, was sterilized in molten metal. As soon as it was sufficiently cool to avoid burning, the point was inserted beneath the gingival margin and the free gingiva was retracted from the tooth to expose the sulcus to view. A blunted right-angled explorer (No. 6, S.S.W.), similarly sterilized, was moistened by dipping into sterile brain-heart infusion broth and given a vigorous shake to prevent any drop from clinging to the point. Avoiding any contact with the tooth or tissue side of the sulcus, the base of the sulcus was gently scraped. The

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point then was introduced into brain-heart infusion broth and Brewer's thioglycolate medium. After 72 hours' incubation, the tubes were examined for growth and the microorganisms isolated for identification.

The maxillary and mandibular incisors and maxillary second molars were chosen for this study. Both the facial and lingual sulci were examined.

RESULTS

To test the accuracy of the technics, a control was used for each test. The explorer employed to collect material was sterilized, dipped into culture media, shaken, and inserted into the culture tubes. After incubation, the tubes showed consistent sterility.

Eighty-six examinations were made of 78 sulci in 33 patients. Of the sulci examined, 75 were on the facial side, 11 on the lingual. Ninety-three per cent showed growth. The details appear in Table I.

TABLE I
BACTERIOLOGIC EXAMINATION OF 86 GINGIVAL SULCI

SOURCE	GROWTH	NO GROWTH	TOTAL	PERCENTAGE SHOWING GROWTH
Controls	0	86	86	0
Adults (healthy)	47	2	49	95.9
Adults treated for periodontal disease	9	1	10	90.0
Children	24	3	27	88.8
Totals	80	6	86	93.0

Identification was made of the organisms recovered from 24 sulci. The results appear in Table II.

TABLE II
IDENTIFICATION OF ORGANISMS FROM 24 GINGIVAL SULCI*

ORGANISM	FREQUENCY OF OCCURRENCE
Lactobacilli	2
Diplococcus pneumoniae	4
Neisseria catarrhalis	13
Pneumococci	4
Sarcina lutea	2
Staphylococcus albus hemolyticus	4
Streptococcus alpha hemolyticus	12
Streptococcus hemolyticus	2
Streptococcus beta hemolyticus	2
Yeasts	12

*Identification by Herbert M. Cobe, Ph.D.

DISCUSSION

The free marginal gingiva does not seal off the sulcus, but lies against the tooth. Hence, it is reasonable to believe that saliva with its accompanying bacteria has free access to the sulcus.

The possibility of contamination by the retracting instrument must be considered since it touches the tooth and the gingival margin on its insertion. The

portion which entered the sulcus was between 0.5 and 1.0 mm. in length. We were unable to measure it more accurately. The sulci selected were more than 1.0 mm. in depth (usually 1.5 to 2.0 mm.) so there was an untouched space below the retractor edge. The material was obtained from the deepest part of the sulcus and it is highly improbable that bacteria could traverse this distance in the few seconds elapsing between retraction by the assistant and the collection of the material. There was not sufficient fluid on the culturing point to make contact with the retractor, no fluid being visible on the point. The point was moistened to ensure adhesion of inoculum. A dry smooth steel point is not the ideal instrument with which to obtain cultures.

These findings do not confirm those of Waerhaug and Steen.[1] The different results are probably due to their method for sterilization of teeth and gingivae. Glycerin cannot be prevented from "creeping," especially when dried by blasts of air, as in their technic. To ascertain the validity of this assumption, 16 sulci were examined after the surrounding tissues were painted with an iodo-glycerol solution consisting of zinc iodide 15 parts, iodine crystals 8 parts, glycerin 50 parts, water 10 parts.

A minimum amount of this solution was applied to the teeth and gingivae, including the gingival margin as advocated by Waerhaug.[2] The solution was carefully dried. Of the 16 sulci so examined, 12 (75 per cent) were sterile. Four (25 per cent) showed growth, perhaps the result of lack of vigorous application when painting the gingival margin, as we feared introduction of iodine into the sulcus. After obtaining the cultures, a supersaturated starch solution was introduced into the sterilized sulcus. The telltale blue iodine-starch reaction could be seen under the magnifying lens.

The flora of other mammalia has been reported.[3] Examination of 50 dogs, 8 cats, 2 lion cubs, 28 pigs, 2 horses, 2 elephants, 1 rhinoceros, 1 monkey, and 2 chimpanzees revealed the paucity of oral micoorganisms in these healthy animals and the difficulty of implanting those bacteria common to man.

In studies of ecology, it has been demonstrated that the qualitative and quantitative characteristics of a biota are in dynamic equilibrium.[4] Without a concomitant environmental change, introduction of other bacteria results in their elimination. Bacteria artificially introduced into the gingival sulcus cannot ordinarily maintain themselves. The bacterial world is strongly antagonistic to changes in the established order. The body defense mechanisms resist the invaders. In other mammals, a totally different environment must be overcome; the body temperature is higher; the saliva has a different composition, a higher pH, and increased antibacterial properties.

SUMMARY

The healthy gingival sulcus is not sterile. In 86 examinations of clinically healthy gingival sulci, bacteria were present in 95.9 per cent of orally healthy adults, 90 per cent of adults at the successful conclusion of periodontal treatment, and 88.8 per cent of children.

Identified organisms in the order of the frequency of their occurrence were *N. catarrhalis, Str. alpha hemolyticus,* yeasts, pneumococci, *Staph. albus hemolyticus, Dip. pneumoniae,* lactobacilli, *Str. hemolyticus, Sar. lutea, Str. beta hemolyticus.*

Sterilization of the teeth and gingivae with iodo-glycerol solution sterilizes the sulcus also.

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THE RESPONSE OF ADULT RAT ORAL TISSUES TO PROTEIN DEPLETION: HISTOLOGIC OBSERVATIONS AND NITROGEN ANALYSIS

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HISTOLOGIC studies of oral tissue responses to varying degrees of protein depletion have been made by several groups of workers. All found oral tissue changes that may be classified as interferences with growth and repair, degenerative changes, and decreased resistance to injury and infection.[1-6] Only Goldman[5] and Stahl, Sandler, and Cahn[6] studied changes in adult animals. Since the methods of evaluating oral tissue responses to protein deprivation in the above studies were primarily histologic, it was desirable to obtain additional data of a biochemical nature. A first step toward this end is the present correlated study of changes in histology, organ weight, and nitrogen content of some oral tissues, as a result of protein depletion in large adult, male, white rats.

METHODS

Thirty male Wistar rats of 320 Gm. average weight were divided into 3 groups of 10 animals each. Group I received a protein-free diet, Group II was pair-fed an isocaloric 18 per cent casein diet, and Group III received the isocaloric 18 per cent casein diet ad libitum. The complete diet contained 18 per cent casein, 13.4 per cent sucrose, 20.2 per cent glucose, 18.2 per cent dextrin, 24.2 per cent lard, 1.7 per cent salt mix (Wesson), 1.0 per cent cod liver oil, 3.3 per cent agar, and the following vitamins (mg. per 2,400 Gm. hydrated agar diet):

Thiamine	4.8	Pyridoxine	3.9
Riboflavin	7.7	Choline	2,400.0
Nicotinic acid	96.0	2-Methyl naphthoquinone	0.5
Calcium pantothenate	96.0	Biotin	0.5
Para-aminobenzoic acid	96.0	Folic acid	0.5
a-tocopherol	10,000.0	Inositol	240.0

The protein-free diet contained 38.2 per cent glucose and all of the above components in the quantities listed, except that casein was omitted. The agar was dissolved in an amount of heated water equal to 1.4 times the weight of the dry constituents. Lard was added and the mixture cooled slightly. The dry ingredients were mixed in by the use of a mechanical stirrer. The slightly warm diet was poured into pans to cool and gel. The food was kept under refrigeration. Water was supplied ad libitum for 62 days. Animals were

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then anesthetized, blood was removed by heart puncture, and the rats were sacrificed. The following organs were dissected out, trimmed, blotted, and weighed: submaxillary and sublingual glands as a unit, one half the mandible with teeth in situ, heart, liver, and kidney. In the case of the paired organs, those of the opposite side were immediately placed in 10 per cent formalin, as were specimens of tongue, heart, and liver. Measurements of mandibular dimensions were made with a Boley gauge. The nitrogen content of compact, marrow and periosteum-free, mandibular alveolar bone, and that of the submaxillary-sublingual gland units was determined by digestion and nesslerization. Statistically significant differences were established using the method of critical ratios.[7]

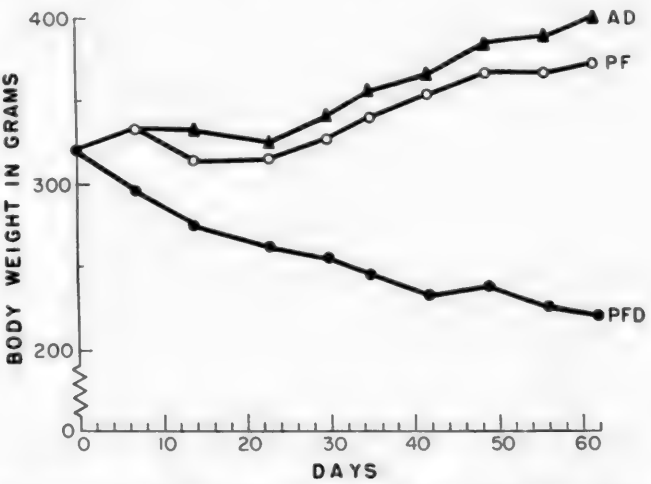


Fig. 1.—Graph showing body weight of animals versus time. PFD–protein-free diet group (I). PF–pair-fed group (II). Ad–ad libitum-fed group (III).

RESULTS

1. *Gross Appearance and Body Weight.*—

The appearance of rats in pair-fed and ad libitum control groups was typical of large, adult, male animals. The protein-depleted animals were smaller and cachectic in appearance. They were otherwise alert and moved nimbly. Skin lesions were not seen in the depleted rats, but their fur was matted and damp in appearance; it tended to clump and resembled very much the phenomenon of "stareing" of head hair seen in human malnutrition.

Fig. 1 shows curves of body weight versus time during the course of the experiment. Depleted animals weighed 46 per cent less than ad libitum controls at the end of the experiment. Pair-fed controls, however, weighed only 7 per cent less than ad libitum animals. This indicates that in adults of this size, paired feeding does not penalize as severely, insofar as body weight is concerned, as is the case with younger, actively growing animals.[2-5]

2. *Mandibular Changes.*—

a. *Weight changes and nitrogen content:* Fig. 2 shows how mandibular length was measured along line *b* and the area *a* from which lingual alveolar

bone was taken for chemical analyses. Results of these measurements show that insofar as length, wet weight, dry weight, and nitrogen content were concerned, there were no significant differences between pair-fed and ad libitum fed groups; the differences all being less than 1 standard deviation for the measurements involved. There were, however, small but statistically significant differences between depleted animals and the control groups for length, wet weight, and dry weight. (In each instance the critical ratios between the control and depleted groups was greater than 2, indicating statistical significance.) Thus, the mean length of control mandibles (ad libitum and pair-fed) was 3.28 ± 0.05 cm., while that of depleted mandibles was 3.09 ± 0.05. The values for ad libitum, pair-fed and depleted mandibular wet weights, respectively, were 0.408 ± 0.021 Gm., 0.409 ± 0.018 Gm., 0.377 ± 0.017 Gm. For dry weights, control mandibles averaged 0.368 Gm. against 0.339 Gm. for depleted animals. There were no significant differences in the per cent nitrogen content of alveolar bone between any of the 3 groups (3.90, 3.80, and 3.89, respectively).

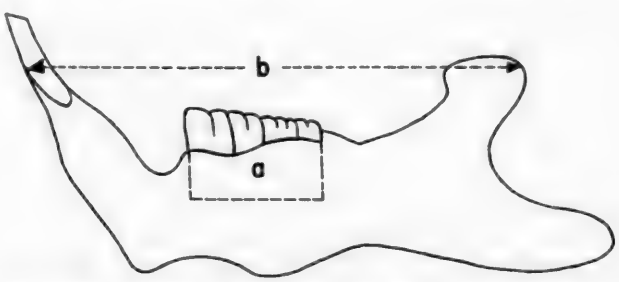


Fig. 2.—Diagrammatic sketch of mandible. The line *b* runs between the posterior-most portion of the condyle and the anterior-most tip of the mandible. The area *a* represents lingual cortical bone overlying the roots of the molars.

b. *Histologic observations:* Histologically, while there was much individual variation, there were no detectable differences between the mandibles of animals from the 3 groups. All showed an osteoporosis which was quite severe and will be discussed later. The periodontal structures were also similar in all groups.

3. *Submaxillary and Sublingual Gland Changes.*—

a. *Weight and nitrogen content:* In Table I is given a summary of submaxillary and sublingual gland changes. There were no differences between ad libitum and pair-fed groups for wet weight, dry weight, total nitrogen, and per cent nitrogen determinations. Significant differences were found between depleted and control groups for wet weight, dry weight, and total nitrogen content of the glands. Thus, for each of the above measurements, the mean values for depleted glands was less than 50 per cent of the mean values for control glands. Insofar as per cent nitrogen was concerned, however, there were no differences between any of the 3 groups. These data demonstrate that the size of the glands of depleted animals was severely affected by lack of protein in the diet, while the composition of the glands, in terms of per cent nitrogen, was unaffected.

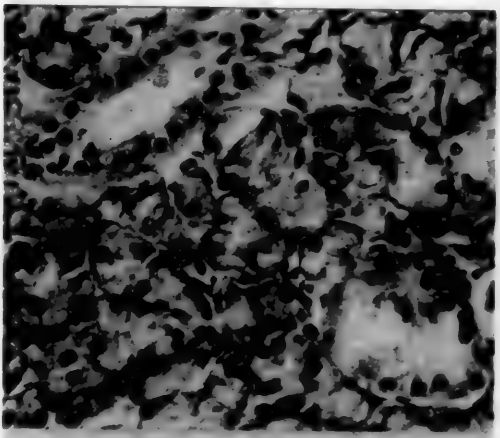


Fig. 3.

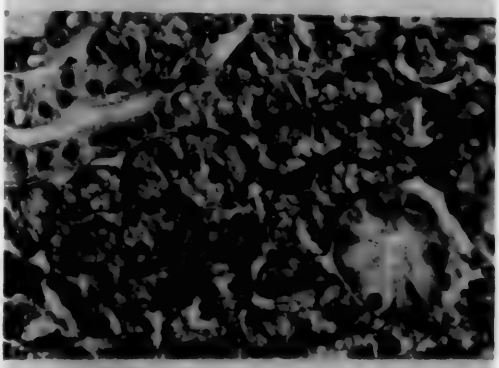


Fig. 4.

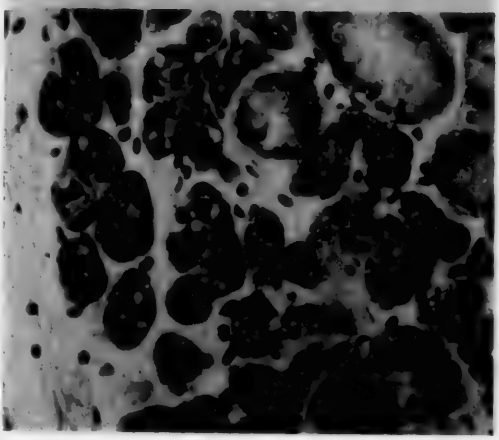


Fig. 5.

Figs. 3, 4, and 5.—Fig. 3 (top) is a section of submaxillary gland from an ad libitum-fed animal and presents typical duct and acinar architecture. Fig. 4 (middle) is a section from a pair-fed animal and is very similar to the above. Fig. 5 (bottom) is a section from a protein-depleted animal showing smaller size of acini and ducts and the spaces between the units, indicative of edema.

TABLE I
SUBMAXILLARY AND SUBLINGUAL GLAND CHANGES
(MEAN VALUES ± STANDARD DEVIATIONS)

GROUP	WET WEIGHT (GM.)	DRY WEIGHT (GM.)	TOTAL N_2 (GM.)	% N_2 CONTENT
Ad libitum	0.273 ± 0.026	0.070 ± 0.004	0.008 ± 0.001	11.85 ± 0.25
Pair-fed	0.281 ± 0.040	0.066 ± 0.011	0.008 ± 0.001	12.25 ± 0.45
Protein-depleted	0.129 ± 0.048	0.027 ± 0.010	0.003 ± 0.001	11.93 ± 1.00

b. *Histologic observations:* The above data are mirrored histologically. Figs. 3, 4, and 5 show the appearance of glands from ad libitum, pair-fed and control animals, respectively. In Fig. 3, the acinar and duct architecture of the glands of ad libitum-fed animals is typical. Fig. 4, a section from pair-fed animals, shows that their glands were similar in appearance. Fig. 5, from depleted animals, shows shrinkage in the size of the acini and tubules with spaces between their borders indicative of edema.

4. *Tongue Changes.*—

For the tongue, histologic data only are given at this time since dissections were not sufficiently definitive for quantitative purposes. Figs. 6, 7, and 8 show representative sections of the posterodorsal surface of tongues of ad libitum, pair-fed, and depleted animals, respectively. Fig. 6 shows the typical appearance of the papillae, epithelium, musculature and glands, and Fig. 7, from a pair-fed animal, shows similar structures with a slight decrease in the thickness of epithelium. Fig. 8, from the depleted group, shows a further decrease in the height of the papillae and epithelium, and shrinkage in size of the gland alveoli is evident.

5. *Nonoral Tissues (Heart, Liver, and Kidney).*—

a. *Weight changes:* In Table II are presented data for the wet weights of heart, kidney, and liver from the 3 groups of animals. There were no significant differences between ad libitum and pair-fed groups. While heart and kidney from depleted animals were almost half the weight of organs from control animals, there were no significant gross liver weight changes in any of the groups.

TABLE II
NONORAL TISSUE CHANGES (WET WEIGHT-GRAMS)
(MEAN VALUES ± STANDARD DEVIATIONS)

GROUP	HEART	KIDNEY	LIVER
Ad libitum	1.025 ± 0.092	1.076 ± 0.120	8.254 ± 1.430
Pair-fed	0.931 ± 0.094	1.030 ± 0.107	8.421 ± 1.552
Protein-depleted	0.675 ± 0.107	0.631 ± 0.089	7.473 ± 1.929

b. *Histologic observations:* Paradoxically, the histologic picture was reversed. In heart and kidney, there were no differences between sections from any of the 3 groups, although weight changes did exist. In the case of protein-depleted liver, which did not differ from control livers insofar as weight is concerned, there was a marked fatty metamorphosis as seen in Figs. 9 and 10. Fig.

9 is representative of ad libitum and pair-fed livers and shows typical liver cord cells and sinusoids. Their appearance may be compared with that shown in Fig. 10 showing a section representative of depleted animals, and demonstrating the fatty metamorphosis.

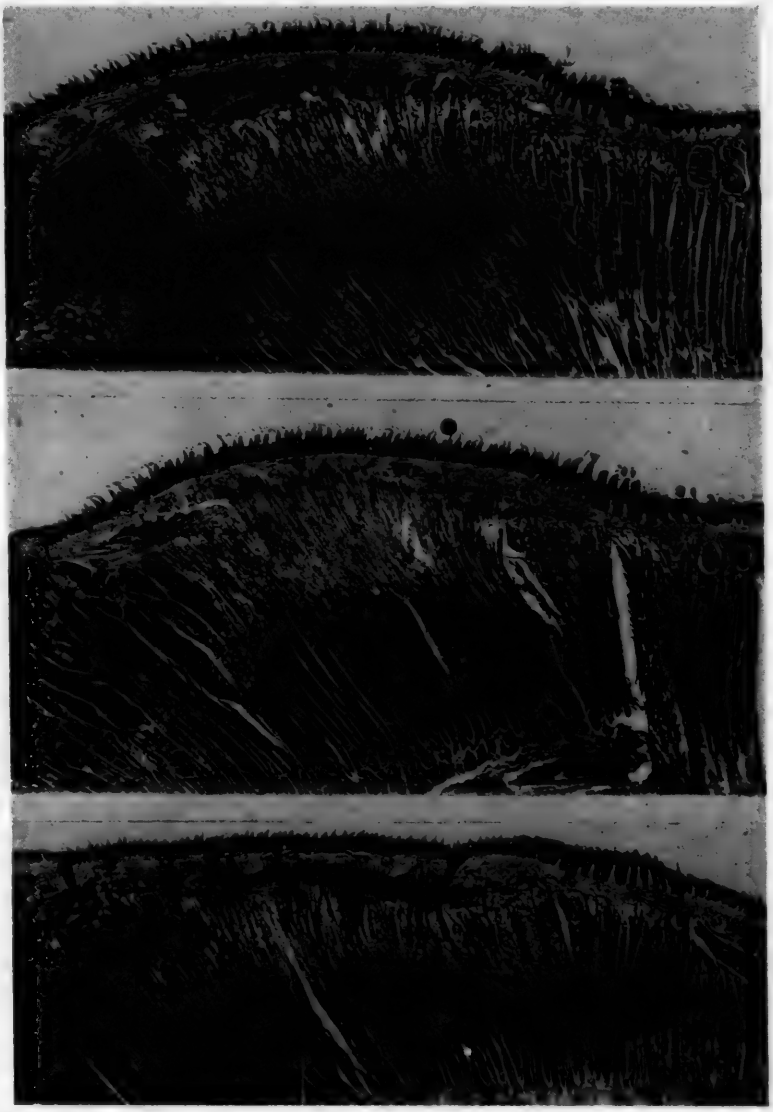
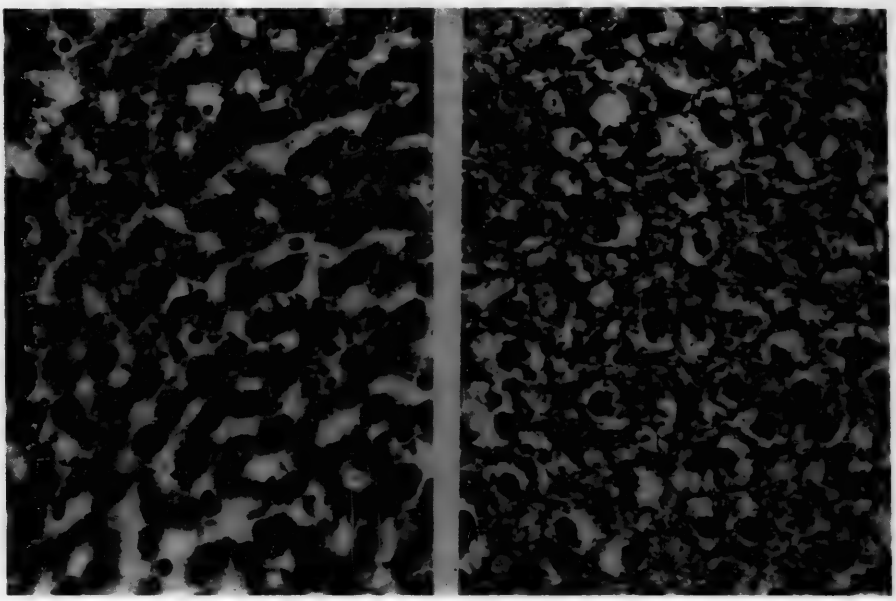


Fig. 6.

Fig. 7.

Fig. 8.

Figs. 6, 7, and 8.—Fig. 6 (top) is a section showing the posterodorsal surface of the tongue of an ad libitum-fed animal. Note the thickness of epithelium, configuration of papillae, and size of the glands; all of which are typical in appearance. Fig. 7 (middle) is a section from a pair-fed animal and is very similar to the above. In Fig. 8 (bottom) may be seen a decrease in thickness of the epithelium, decrease in height and numbers of papillae, and decrease in size of the glands of the posterior of the tongue.



Figs. 9 and 10.—Fig 9 (left) is a section representative of the livers of ad libitum and pair-fed rats. The typical liver cell cords and sinusoid are seen. In Fig. 10 (right) the severe fatty metamorphosis of the depleted livers is shown.

DISCUSSION

From the measurements and histologic data presented, it is evident that protein depletion in large, adult, male rats causes less drastic oral tissue changes than are seen in young, actively growing, animals. These observations confirm and extend the histologic observations of Goldman,[5] who studied the periodontal structures of old and young rats during protein depletion, and also those of Stahl, Sandler, and Cahn,[6] who studied periodontal structures, femur, tongue, and cheek mucosa in adult rats during protein depletion. In neither of the above reports, however, was a comparable degree of osteoporosis noted in non-depleted animals. The severe osteoporosis in ad libitum and pair-fed animals, as well as the depleted ones, is striking. We have been unable to find in the literature,[8-11] reference to a similar degree of osteoporosis in the alveolar bone of old rats. It is interesting to note, however, that Baer and Bernick,[12] in a recent study of ageing of the periodontal structures in mice, reported that in old mice there was a similar alveolar bone picture to that encountered in the present investigation. It may be that genetic factors in our animals were responsible. It is also possible that the physical character of the diet, which is prepared in an agar base and is of a gelatin-like consistency, may be responsible. The rats are very fond of this diet and are able to bite and bolt it, without chewing it thoroughly. The extent to which the above-mentioned physical factors may have been responsible for the osteoporosis is now under study in our laboratory.

For purposes of comparison of the effects of protein depletion upon the various oral and nonoral tissues studied, reference may be made to Fig. 11, a graphical summary of the weight and nitrogen determinations, presented in the last section. Additional data relating to per cent nitrogen content of heart, kidney, and liver have been included in this graph in order to permit a more complete comparison. These data were taken from an experiment performed in identical manner to the present one, in which animals of similar

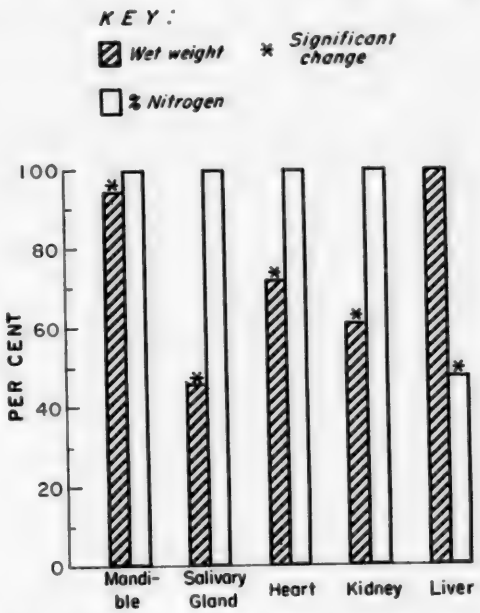


Fig. 11.—Wet weight and nitrogen determinations of depleted animal organs expressed as per cent of pair-fed control values. The asterisks indicate significant differences between depleted and control tissues as determined by the method of critical ratios (if critical ratio is equal to or greater than 2, differences are significant.)

body weights were used. It may be seen that, insofar as size (wet weight) of organs is concerned, the salivary glands were most severely affected by protein depletion. With the exception of liver, the per cent composition of the tissues was unaffected by depletion. The reason that total liver weights did not reflect the loss in nitrogen content is that the increase in fat content made up for nitrogen loss and, in some instances, may have exceeded it.

SUMMARY

A study has been made of the responses of oral tissues of large, adult, male rats to protein depletion during a 63-day period. Histologic changes were compared and correlated with organ weight and nitrogen content changes. The oral structures studied were submaxillary and sublingual glands, mandible, and tongue (histologic study only). The responses of these structures were compared with those of heart, liver, and kidney.

Results were as follows:

1. Weight Changes. (a) Protein-depleted animals weighed 46 per cent less than ad libitum-fed control animals. Food restriction, caused by depletion, accounted for only 7 per cent of this difference, as shown by pair-fed controls. (b) All organs studied weighed less in depleted animals than in controls, with the exception of liver. Greatest weight loss occurred in the salivary glands.

2. Nitrogen Content. Although organs from depleted animals were smaller and contained less total nitrogen than organs from controls, the per cent nitrogen content was the same in all tissues of all groups, with the exception of liver. Livers from depleted animals weighed approximately the same as livers from controls, and had less nitrogen and more fat than control livers.

3. Histology. (a) Sections through the lower first molar and its periodontium revealed marked osteoporosis in all groups of animals. (b) The salivary gland acini and ducts were smaller in depleted animals than in controls. Spaces, indicative of edema, were seen between borders of adjacent acini. (c) The tongue changes were of a mild atrophic nature and affected epithelial thickness, papillae, and glands of the tongue. (d) The histologic appearance of heart and kidney was unchanged in depleted animals, while liver of depleted animals showed an extensive fatty metamorphosis.

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SEMIQUANTITATIVE STUDIES OF IN VITRO CARIES BY MICROHARDNESS TESTS

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THE purpose of the present investigation was to develop a method for measuring the rate of in vitro tooth decay. Since surface softening has been one of the initial signs of in vitro tooth decay, changes in hardness of the enamel surface might be a useful index of the rate of the caries attack. The value of a rapid quantitative method for evaluating anticaries agents is readily apparent.

The "artificial mouth"[1] has been used in this laboratory to produce in vitro tooth decay, the carious lesions produced being indistinguishable from those occurring in vivo. This has been shown by the general similarities[1] and by histologic,[2] ultraviolet and polarized light[3] studies of the lesions produced.

The microhardness of intact surface enamel has also been investigated in this laboratory.[4] It was shown that intact surface enamel varies in hardness on a single tooth surface as well as from tooth to tooth. The variation in hardness is generally within the range of 250 to 450 Knoop numbers. The inherent variation in hardness of a single surface poses a real problem when only slight changes in hardness are being measured. However, if a tooth is softened so much that its average hardness no longer falls within the normal range, then a definite softening effect will be demonstrable.

EXPERIMENTAL

Fig. 1 is a diagram of the apparatus used in the semiquantitive study of in vitro caries. The crowns of caries free, human, anterior teeth were cut into mesial and distal halves. The two halves were mounted, labial surface up, in an acrylic resin box (*A*) and held securely in place with inlay wax. Ten microhardness measurements were made on each half and the average hardness calculated for each tooth half.

The tooth halves were then subjected to a carious attack in the "artificial mouth" for 6 to 14 hours and their hardness redetermined. Bacteriologic medium, the same as used in previous work,[5] containing 0.25 per cent glucose was maintained at a constant drip rate of 60 drops per minute. The medium was dripped onto a cotton muslin cloth (*B*) through which it slowly drained.

Since some bacterial plaques made in the laboratory tend to develop faster than others, all plaques were pregrown for 24 hours in the "artificial mouth"

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before placing them on the tooth. The plaques were prepared by placing a double thickness of the cloth (*B*) on top of an acrylic resin box identical to those in which the teeth were mounted. The top section (*C*) was placed over the cloth and held in place with a rubber band looped around the box and top. The purpose of the top section was to prevent the medium from draining off immediately, allowing an even growth of bacteria to be produced under a relatively constant volume of medium. The cloth was first soaked by pipetting over it 4 ml. of distilled water and was then inoculated with 4 ml. of pooled saliva collected 1 hour after eating. After the saliva had drained through the cloth, the medium was dripped on the cloth at the rate of one drop every 2 or 3 seconds. After 24 hours the box containing the experimental tooth was substituted for the one used for preparing the plaque. The cloth was inverted on the tooth with the plaque in contact with the enamel surface and the drip rate increased to 1 drop per second.

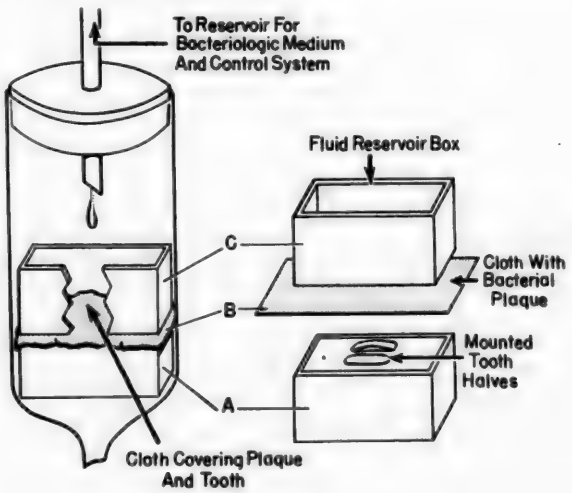

Fig. 1.—Apparatus for semiquantitative measurement of in vitro caries.

RESULTS

Changes in Hardness as a Result of In Vitro Decay.—The measures outlined above were intended to produce a standard carious attack but it was found that 2 halves of the same tooth mounted side by side would frequently soften at different rates. Certain definite trends became obvious, however, when the results from several similarly treated teeth were averaged.

Table I records the average changes in hardness for 52 tooth surfaces as a result of in vitro decay. Thirty-one of these surfaces were intact, whereas 21 were ground. The ground surfaces were prepared by applying progressively finer abrasive papers and finally polishing with a round piece of ordinary stationary paper supported by a smooth-sided disc. The average original hardness (prior to softening in the "artificial mouth") was 367 K. N. for the intact

enamel surfaces. This hardness is in close agreement with our previously published values.[4] The ground subsurface enamel averaged somewhat less, with a hardness of 325 K. N. This result is in agreement with Hodge and McKay's report[6] that enamel decreases in hardness toward the dentinoenamel junction.

TABLE I. AVERAGE CHANGES IN HARDNESS AS A RESULT OF ARTIFICIAL DECAY

TYPE OF SURFACE	NO. OF SURFACES	HARDNESS (K. N.) BEFORE DECAY	HARDNESS (K. N.) AFTER DECAY	DECAY TIME (HR.)	AVERAGE HARDNESS DECREASE (K. N.)	DECREASE IN HARDNESS PER HOUR (% ± S.D.)
Intact	31	367	262	10.3	105	3.4 ± 1.7*
Ground	21	325	170	9.6	155	4.9 ± 1.3*

*Significance of difference ("t" test) $0.005 < p < 0.01$.

After the decay period in the "artificial mouth," the intact enamel surfaces had decreased in hardness to 262 K. N. and the ground enamel had decreased to 170 K. N. The decay period in the "artificial mouth" averaged 10.3 hours for the intact surfaces and 9.6 hours for the ground surfaces. The average decrease in hardness was 105 K. N. for the intact surfaces and 155 K. N. for the ground surfaces even though their decay period was slightly shorter.

To put these results on a comparable basis, they were recalculated to the percentage decrease in hardness per hour. For the intact surfaces, the rate of softening was 3.4 ± 1.7 (S.D.) per cent per hour, corresponding approximately to a 35 per cent reduction in hardness for the total time period. The ground surfaces had a greater measurable softening of 4.9 ± 1.3 (S.D.) per cent per hour. This difference in percentage decrease in hardness per hour between the intact and ground surfaces was statistically significant at the 0.01 level (Student "t" test). Thus, by this method, it is possible to demonstrate a softening effect as a result of artificial decay; this surface softening is measurable and is greater for ground surfaces than for intact surfaces.

Effect of Fluoride on Enamel Softening.—The method was then used to measure the effect of an anticaries agent. Since it has been well established clinically that fluoride retards decay, it was selected as a known anticaries agent. Table II records the effect of fluoride treatment on the retardation of artificial decay. The change in tooth hardness for both intact and ground surface enamel was calculated as percentage decrease in hardness per hour.

TABLE II. AVERAGE EFFECT OF FLUORIDE TREATMENT ON RETARDATION OF ARTIFICIAL DECAY

	DECREASE IN HARDNESS AS % PER HOUR				
	UNTREATED		TREATED		
TYPE OF SURFACE	NO. OF SURFACES	AVERAGE % DECREASE	NO. OF SURFACES	AVERAGE % DECREASE	SIGNIFICANCE OF DIFFERENCE IN AVERAGES*
Intact	31	3.4 ± 1.7	12	2.5 ± 1.2	$.05 < p < .10$
Ground	21	4.9 ± 1.3	12	3.3 ± 2.0	$.005 < p < .01$

*Student "t" test.

The fluoride treatments were made with sodium fluoride at 0.2 per cent and 2.0 per cent concentration, both unbuffered and buffered at pH 6.2. Stannous fluoride was used at 0.4 per cent concentration. In some cases the fluoride

was applied by swabbing the tooth surface 3 times, allowing it to dry between each application. Other methods of applying the fluoride were by mechanically stirring the teeth in the fluoride or soaking the teeth overnight in the solution.

Phillips and Swartz[7] have reported that enamel is hardened by fluoride treatments. In the present work the fluoride treatments of enamel had very little hardening or softening effect. Previously the variability in the results of hardness testing was discussed.[4] These sources of error, in addition to those arising from the inherent variations in hardness of enamel in different areas of the same enamel surface, suggest that small changes in the hardness of intact surface enamel following fluoride treatments may be due to the problems in measuring enamel hardness. In the present work, no demonstrable changes in enamel hardness due to fluoride treatments could be found which were greater than the experimental error of testing.

The 31 intact surfaces not treated with fluoride had an average decrease in hardness of 3.4 per cent per hour, as compared with 2.5 per cent per hour for a group of 12 intact surfaces which received fluoride treatment. The significance of this difference in the averages for the treated and untreated intact surfaces, determined by the Student "t" test, revealed only a questionable effect of fluoride treatment upon the rate of softening of intact surfaces ($0.05<p<0.1$). For the ground surfaces, the untreated group showed an average decrease of 4.9 per cent per hour as compared to 3.3 per cent per hour for the fluoride-treated group. This difference was found to be statistically significant ($0.005<p<0.01$). Thus for ground surfaces, the method reflects a definite difference in softening rate between those surfaces which were fluoride treated and those left untreated.

Often the enamel surface would become measurably softer after decaying, although no surface changes were visible to the unaided eye. During the initial work, longer decay periods were used and frequently a chalkiness of the surface was observed with the unaided eye. When examined with the microhardness tester, the microindentations from the original hardness tests were completely gone. This change indicated a loss of surface as well as a softening. For the shorter decay periods the original hardness testing indentations could still be seen, but would be slightly obliterated or shortened. Thus it appears that for periods of 6 to 14 hours the effect of the decay is primarily that of surface softening while for longer periods, surface removal becomes increasingly apparent.

DISCUSSION

Surface softening resulting from in vitro decay can be measured semiquantitatively by means of hardness tests. With the method in its present stage, a measurable retarding effect of fluoride on softening of ground surface enamel could be demonstrated. The effect of fluoride was not so clearly demonstrable upon intact surface enamel.

Because of the variability of the attack on single surfaces, it was necessary to average the results from many teeth in order to establish trends. However, the method shows promise. With further refinements, a more rapid

method may be developed whereby it is possible to evaluate anticaries agents by using a small number of teeth. Further study directed toward this is now in progress.

SUMMARY

1. A semiquantitative method for measuring the rate of in vitro caries was developed. The method needs further refinement before it will be useful for routine work.

2. The effect of fluoride in retarding in vitro decay was measurable by changes in enamel hardness.

3. Intact enamel surfaces were not so obviously protected by fluorides as were ground polished enamel surfaces.

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CARIES SUSCEPTIBILITY TESTS, HYALURONIDASE ACTIVITY OF SALIVA AND DENTAL CARIES EXPERIENCE

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THE factors in saliva most frequently related to dental caries are (a) aciduric and acidogenic bacteria[1-12] and (b) the rate of acid production in the presence of glucose.[13-17] Other factors which have been suggested as being related to dental caries include (c) the amount of saliva secreted in a given time[18-21] and (d) the acid neutralizing ability (buffering capacity) of saliva.[22-25]

Included within the studies of saliva at this laboratory were the following tests, performed simultaneously on a number of subjects: (1) rate of flow of stimulated saliva, (2) pH of saliva sample, (3) acid-neutralizing ability of saliva sample, (4) lactobacillus population of saliva, (5) acid-producing ability of saliva in a glucose media, and (6) hyaluronidase activity of saliva. Inasmuch as the subjects involved in these tests consisted of young adults living in the same environment under the same conditions, a certain uniformity of external factors was possible.

The purpose of this report is to demonstrate the relationship of the above characteristics of the saliva of naval recruits to dental caries experience and to salivary hyaluronidase activity.

METHOD OF STUDY

The subjects for this study were naval recruits undergoing training at the U.S. Naval Training Center, Bainbridge, Md., from January, 1955, to February, 1956. Both random and purposive selection of individuals were used in order to obtain a test group with an adequate distribution of dental conditions for analysis. A total of 1,049 subjects were included in the test group, 75 of whom had no carious teeth at the time of the test. This latter group included 63 individuals who showed no evidence of dental caries or of dental caries experience.

An oral examination using a mouth mirror, explorer, and adequate lighting, supported by bite-wing radiographs interpreted with the aid of a 10× magnifying glass was the method used for diagnosing dental caries. The number of DMF (decayed, missing, and filled) teeth, the number of carious teeth, and the number of carious surfaces were determined and tabulated in each case.

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Saliva was collected on 3 successive days 1 week apart by paraffin stimulation from naval recruits at 11 o'clock in the morning, after 5 hours of supervised fasting.

The rate of flow of stimulated saliva was determined by recording the length of time necessary to fill a marked tube to 20 ml., the subject starting with a bolus of paraffin in a relatively dry mouth. The rate of flow was computed as the number of milliliters of saliva delivered per minute.

The lactobacillus count of the saliva was measured using the pour-plate technic described by Rogosa, Mitchell, and Wiseman[26] with a standard dilution of 1:100 whole saliva in saline for the inoculum.

The acid-producing ability of saliva was determined by the colorimetric test described by Snyder[14] using 0.2 ml. of whole saliva for each inoculation.

The pH and acid-neutralizing ability of each sample was carried out on 5 ml. of supernatant saliva after centrifuging at room temperature for 15 minutes at 1,200 G. The initial pH of each sample and the titration were carried out on an automatic titrator that is accurate to 0.1 of a pH unit and 0.05 ml. of titrant. 0.05N HCl was used to titrate 5 ml. of sample to a pH of 4.4.

All of the above tests were completed (or inoculated) within 4 hours of the time the saliva was collected. During this time the saliva was either in process or refrigerated at 4° C.

The hyaluronidase activity of saliva was determined on the supernatant using a modified viscosity-reduction technic.[27]

The report presents the median results of tests carried out on 3 samples of saliva for each individual.

For purpose of analysis, 3 groups were selected out of the total number of cases studied. The characteristics of the study groups are listed in Table I. Group A consisted of 63 subjects free from any indication of dental caries experience as verified by 3 examining dentists using both clinical and bite-wing radiographic examinations. Group B was made up of 131 subjects who had 1 to 6 surfaces of caries experience. This group had an average of 3.42 DMF teeth per case with the above interpretation. Group C of 138 subjects is made up of 2 companies of recruits selected at random. This group has an average DMF of 16.0. The average DMF for the entire study group is 13.5 and is listed on Table I (Group D).

TABLE I

CHARACTERISTICS OF STUDY GROUP FOR EVALUATION OF SELECTED TESTS OF SALIVA USNTC, BAINBRIDGE, 1955-1956

ITEM	GROUPS			
	A	B	C	D
Number of cases	63	131	138	1,049
Average age per case	19	18.8	19.3	18.5
Average number of teeth per case	29.4	28.8	26.4	26.6
Average DMF per case	0	3.4	16.0	13.5

RESULTS

The summary of the findings distributed according to the selected groups is recorded in Table II. It will be noted that there is no significant difference in the pH, the acid-neutralizing ability or hyaluronidase activity of saliva among the 3 selected groups. However, there is a significant difference in the rate of flow of saliva, Snyder test, and lactobacillus count.

TABLE II
SALIVA CHARACTERISTICS OF NAVAL RECRUITS ACCORDING TO THE SELECTED GROUPS USNTC, BAINBRIDGE, 1955-1956

GROUPS	NO. OF CASES	AVERAGE TEST RESULTS					
		RATE OF FLOW[1]*	pH[2]	ACID-NEUT. ABILITY[3]	SNYDER TEST[4]*	LACTOBACILLUS COUNT[5]*	HYALURONIDASE ACTIVITY[6]
A	63	2.96	7.88	2.56	0.73	Less than 100	11.48
B	131	2.79	7.91	2.51	1.64	Less than 100	11.29
C	138	2.18	7.96	2.53	2.51	5,000 to 9,999	12.53

[1]Number of milliliters of saliva delivered per minute.
[2]pH of saliva supernatant.
[3]Number of milliliters of .01N HCl required to titrate to pH 4.4.
[4]Values interpreted as 0, 1, 2, 3, or 4.
[5]Number of lactobacilli per milliliter of saliva.
[6]In seconds drop in viscosity of saliva-substrate mixture per 45 minutes.
*Differences statistically significant.

The summary of the findings distributed according to the various classes of numbers of DMF teeth for all cases is recorded in Table III. There is a significant difference in the Snyder test and lactobacillus count between the high and low DMF classes. There is a difference apparent in the rate of flow that is statistically significant but not marked. The acid-neutralizing ability is also progressively different in this distribution. The pH and hyaluronidase activity of saliva is not significantly different in any class.

TABLE III
SALIVA CHARACTERISTICS OF NAVAL RECRUITS ACCORDING TO THE QUANTITATIVE GROUPS OF DMF TEETH FOR CLASSIFYING DENTAL CARIES EXPERIENCE

CLASSIFICATION OF DMF	NUMBER OF CASES	AVERAGE RESULTS					
		RATE OF FLOW[1]	pH[2]	ACID-NEUT.[3]	SNYDER TEST[4]	LACTOBACILLUS COUNT[5]	HYALURONIDASE ACTIVITY[6]
0	63	2.91	7.93	2.55	0.73	Less than 100	11.3
1-4	94	2.72	7.94	2.49	0.92	Less than 100	11.3
5-9	131	2.75	7.93	2.50	1.99	1,000 to 4,999	11.4
10-19	546	2.55	7.92	2.38	2.71	10,000 to 24,999	10.7
20-28	215	2.50	7.89	2.34	3.13	25,000 to 49,000	10.4
All Cases	1,049	2.60	7.92	2.41	2.43	10,000 to 24,999	10.8

[1]Number of milliliters of saliva delivered per minute.
[2]pH of saliva supernatant.
[3]Number of milliliters of .01N HCl required to titrate to pH 4.4.
[4]Values interpreted as 0, 1, 2, 3, or 4.
[5]Number of lactobacilli per milliliter of saliva.
[6]In seconds drop in viscosity of saliva-substrate mixture per 45 minutes.

The summary of the findings distributed according to the various classes of numbers of carious teeth for all cases is recorded in Table IV. The Snyder test and lactobacillus count are markedly different between the high and low

classes and the rate of flow and acid-neutralizing ability are slightly different while the pH of the saliva sample and the hyaluronidase activity is not different in any class.

TABLE IV
SALIVA CHARACTERISTICS OF NAVAL RECRUITS ACCORDING TO THE QUANTITATIVE GROUPS OF CARIOUS TEETH FOR CLASSIFYING DENTAL CARIES EXPERIENCE

CLASSIFICATION OF CARIOUS TEETH	NUMBER OF CASES	AVERAGE RESULTS					
		RATE OF FLOW[1]	pH[2]	ACID-NEUT.[3]	SNYDER TEST[4]	LACTOBACILLUS COUNT[5]	HYALURONIDASE ACTIVITY[6]
0	75	2.90	7.97	2.52	0.89	100-999	10.7
1-4	168	2.65	7.99	2.47	1.48	100-999	11.1
5-9	343	2.58	7.97	2.41	2.47	5,000 to 9,999	10.6
10-19	434	2.55	7.96	2.36	2.96	25,000 to 49,000	10.9
20-32	29	2.73	7.94	2.43	3.41	25,000 to 49,000	10.6
All Cases	1,049	2.60	7.92	2.41	2.43	10,000 to 24,999	10.8

[1]Number of milliliters of saliva delivered per minute.
[2]pH of saliva supernatant.
[3]Number of milliliters of .01N HCl required to titrate to pH 4.4.
[4]Values interpreted as 0, 1, 2, 3, or 4.
[5]Number of lactobacilli per milliliter of saliva.
[6]In seconds drop in viscosity of saliva-substrate mixture per 45 minutes.

DISCUSSION

The difference between the high and low dental caries experience groups are found in the lactobacillus count and acid-producing ability (Snyder test). In addition, there is a difference in the rate of flow and acid-neutralizing ability, although not marked, that is statistically significant.

Inasmuch as all of these tests were carried out simultaneously on individual samples of saliva certain characteristics of saliva are possible for interrelating them to dental findings. Three thousand one hundred and forty-seven tests were conducted on each procedure within the study (3 per case per 1,049 cases) thus making a total of 18,882 tests represented in Tables III and IV.

To further evaluate the results, all data (1,049 cases) were distributed according to the classes of DMF and carious teeth (Tables III and IV). The only marked variation occurring in the Snyder test and lactobacillus counts. Although the pH is not significantly different, there is a slight difference in the acid-neutralizing ability of the sample and rate of flow. Hyaluronidase activity is not related to any of the tests conducted or to dental caries experience.

CONCLUSIONS

1. There is a direct relationship of the salivary lactobacillus count to dental caries experience as revealed by the number of DMF teeth and number of carious teeth present in groups at the time of the test. The greater the lactobacillus count the greater the caries experience.

2. There is a direct relationship of the acid-producing ability of saliva in a glucose media as determined by the Snyder test to dental caries experience as revealed by the number of DMF teeth and number of carious teeth in groups present at the time of the test. The greater the acid-producing ability, the greater the caries experience.

3. There is an inverse relationship of the rate of flow of stimulated saliva to dental caries experience in groups.

4. There is an inverse relationship of the acid-neutralizing ability of the saliva sample supernatant after 1,200 G. centrifuging to caries experience in groups.

5. There is no relationship of the pH of the saliva sample supernatant after 1,200 G. centrifuging to caries experience in groups.

6. There is no relationship of hyaluronidase activity of saliva to dental caries experience in groups.

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MASTICATORY STRESSES IN NORMAL AND MODIFIED OCCLUSION

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USING a simple electrical recording device, Anderson and Picton[1] showed, during unilateral chewing of various foods, that the teeth on the empty side of the mouth frequently came into contact. Previous experiments by Anderson[2] had established values for the loads transmitted by a tooth on which food was being chewed, and the first of the series of experiments to be reported here was designed to enable a comparison to be made between the loads on the chewing and empty sides of the mouth. Such measurements were made with the object of estimating the load distribution over the whole dental arch. If whole-arch contact and even distribution of load were normal features of masticatory behavior, then, from a purely mechanical standpoint, reduction of the load-bearing area to one or two teeth with the same total over-all load should greatly increase the pressure on the supporting tissues of the contacting teeth. Such a situation might occur if teeth were lost or came into premature contact due to misplacement, and could be described as potentially traumatic occlusion. A concept of this nature would ignore the possibility of some force-regulating mechanism depending on contact or pressure receptors within the supporting tissues of the teeth and jaws.

In addition to recording the loads on the chewing and empty sides of the mouth, comparisons were also made between the loads recorded on the normal tooth and those recorded when the strain-gauge unit and occlusal surface of this tooth were raised by approximately 0.5 mm. above the general occlusal level. In a third group of experiments, a comparison was made between the loads on the normal tooth and those recorded with the strain-gauge unit, out of occlusion by 0.5 mm. This series was designed to mimic the loading experience of a tooth out of occlusion.

The results of the 3 groups of experiments can be summarized as follows: (1) there is a great similarity between the loads on the chewing and empty sides of the mouth, (2) when the recording occlusal surface was elevated, the loads taken by the tooth appear in some cases to be increased above the normal, although not to the extent to be expected from a simple physical system, and (3) when the recording surface was lowered, the records were markedly changed both qualitatively and quantitatively.

METHOD

Masticatory loads were recorded with the strain-gauge apparatus calibrated for whole-tooth loads according to the technic already described.[2] In order to

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record from the tooth raised above its neighbors and compare the loads with those from the tooth in its normal occlusal relationship, it was necessary to prepare a removable cap which could be fitted to the tooth during the "raised-bite" recording.

The technic of manufacturing this cap was as follows: An impression was taken of the tooth with the strain-gauge apparatus in its normal position, and a model was made in stone. An investment plaster reverse was cast onto this model as shown in Fig. 1, *A*. A second impression of the tooth was taken with the strain-gauge apparatus raised on a platform 0.5 mm. thick and an investment plaster model made from this (Fig. 1, *B*.) Careful positioning of the investment reverse on this model left a space between the reverse and the model over the whole occlusal surface except for the area of contact between them on the strain gauge. The margins of the space were sealed with casting wax and the wax was extended on the buccal and lingual aspects of the model to form a thin cap. From this a hard gold cap was cast, having the same anatomic detail as the strain-gauge tooth, but fitting this tooth and providing it with a new occlusal surface approximately 0.5 mm. above its normal level. Buccal and lingual clasps were soldered on to ensure stability in the mouth.

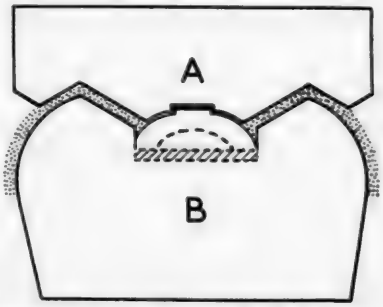

Fig. 1.—Diagram illustrating the construction of a "bite-raising" cap. The solid lines show the outlines of the investment plaster models; the dotted lines show the position of the strain-gauge unit on the raising platform 0.5 mm. thick. The stippled area indicates the extent of the wax pattern of the bite-raising cap.

To determine the loads with the recording apparatus out of contact with the opposing tooth, the strain-gauge unit was reduced in height by approximately 0.5 mm. and greatly reduced in thickness to give greater sensitivity. New covering inlays were prepared to adapt the immediate surroundings of the strain-gauge apparatus to its new level. Preliminary experiments with this strain-gauge unit showed a rapid diminution in load in the early part of the chewing sequence and many thrusts were made without a measurable load. It was therefore necessary to have some indication, other than from the strain-gauge unit, when chewing thrusts were being made and this was obtained by recording the electrical activity from the skin overlying the masseter muscle on the chewing side.

As in previous experiments the subjects were given ½ inch cubes of food which, in this series, was crustless dry bread. In the experiments to compare loads on the chewing side of the mouth with those on the empty side, the subjects chewed alternately one piece of bread on the strain-gauge side and one piece

on the opposite side, using 20 pieces of bread altogether. The experiments were spread over several sessions. In the raised and lowered bite series, 2 or 3 records were made in succession with the strain gauge in the normal position and these were immediately followed by 2 or 3 with the recording apparatus raised or lowered. There is a total of 10 control and 10 test sequences in each series.

The apparatus was calibrated outside the mouth in the usual manner in the experiments to compare loads on the chewing and empty sides. In the raised-bite series, the relationship between the recording tooth and its opponents was established by a wax-bite with and without the occlusal cap in position in order to align the models for calibration. In the lowered-bite series, since the recording surface was out of contact with the opposing teeth, whole-tooth calibration was not possible. The calibration was by direct loading of the strain-gauge apparatus and the comparison between the normal and lowered-bite results is on the basis of strain-gauge loads rather than whole-tooth loads.

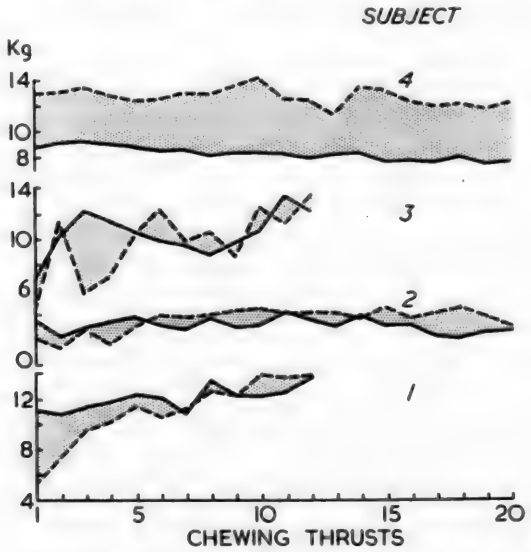

Fig. 2.—Mean whole-tooth loads in kilograms per chewing thrust in 10 sequences from 4 subjects when chewing on the strain-gauge side of the mouth (*dotted lines*), and when chewing on the opposite side of the mouth (*solid lines*).

RESULTS

The treatment of the results was as follows: After determinating the loads applied at each thrust by referring to a calibration curve, the values were plotted on a graph with the individual thrusts evenly spaced on the abscissa. In all groups of experiments, there were records taken at every session with the subjects chewing on the normal strain-gauge tooth for comparison with the results during the particular experimental procedure. The set of curves in Fig. 2 have been drawn to the mean values for each successive thrust in the 10 sequences. Since not all the chewing sequences consisted of the same number of thrusts, the points toward the end of the curves are means of fewer than 10 but not less than

6 values. Some of the sequences were much longer than the rest and the last few thrusts of such sequences were not included in the data from which the curves were drawn.

It is clear from these curves that in Subjects 1, 2, and 3, the loads recorded when chewing took place on the strain gauge are not different from those recorded when chewing took place on the opposite side of the mouth, except for the early part in Subject 1. In Subject 4, the lines are well separated but this difference and the difference in Subject 1 are unlikely to be significant in view of the wide scatter about the means.

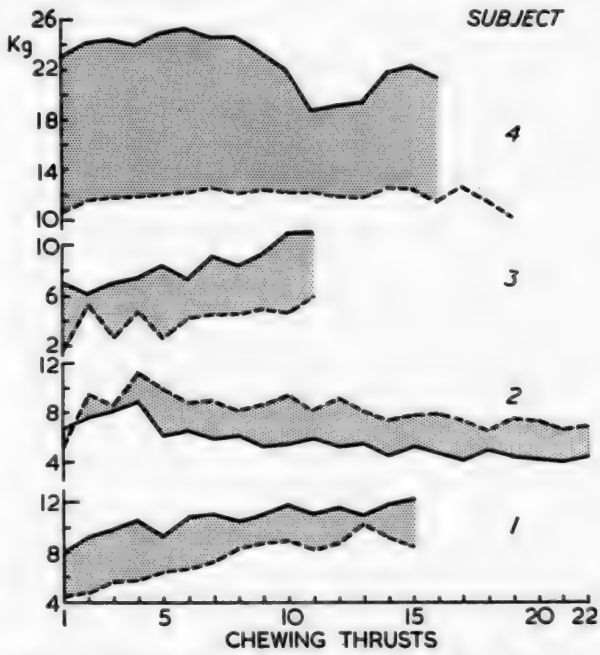

Fig. 3.—Mean whole-tooth loads in kilograms per chewing thrust in 10 sequences from 4 subjects when chewing on the normal strain-gauge tooth (*dotted lines*) and when chewing on this tooth with the bite-raising cap in position (*solid lines*).

Fig. 3 shows curves obtained with the normal and raised-bite data in the manner already described. In all, except Subject 2, the high-bite loads are greater than the normal by amounts which approximate to 100 per cent. In Subject 2, the high-bite loads appear to be consistently less than the normal.

Fig. 4 shows the collected data from Subject 3 in the lowered-bite experiment. Diagram A shows the normal strain-gauge loads in 10 sequences. Diagram B shows the lowered strain-gauge loads in 10 sequences. A similar trend was seen in all subjects.

DISCUSSION

With foods which offer little resistance, such as apple, carrot, crustless bread, and cooked meat, the teeth frequently come into centric occlusal contact on the

empty side of the mouth during unilateral chewing, and the reasons for concluding that this is whole-arch contact have been discussed previously.[1] The fact that this investigation has shown a close similarity between the load patterns on the chewing and empty sides of the mouth supports this conclusion. Furthermore, in the lowered-bite series, the strain-gauge unit and opposing cusp were always out of contact by 0.5 mm. and loads could only be transmitted through

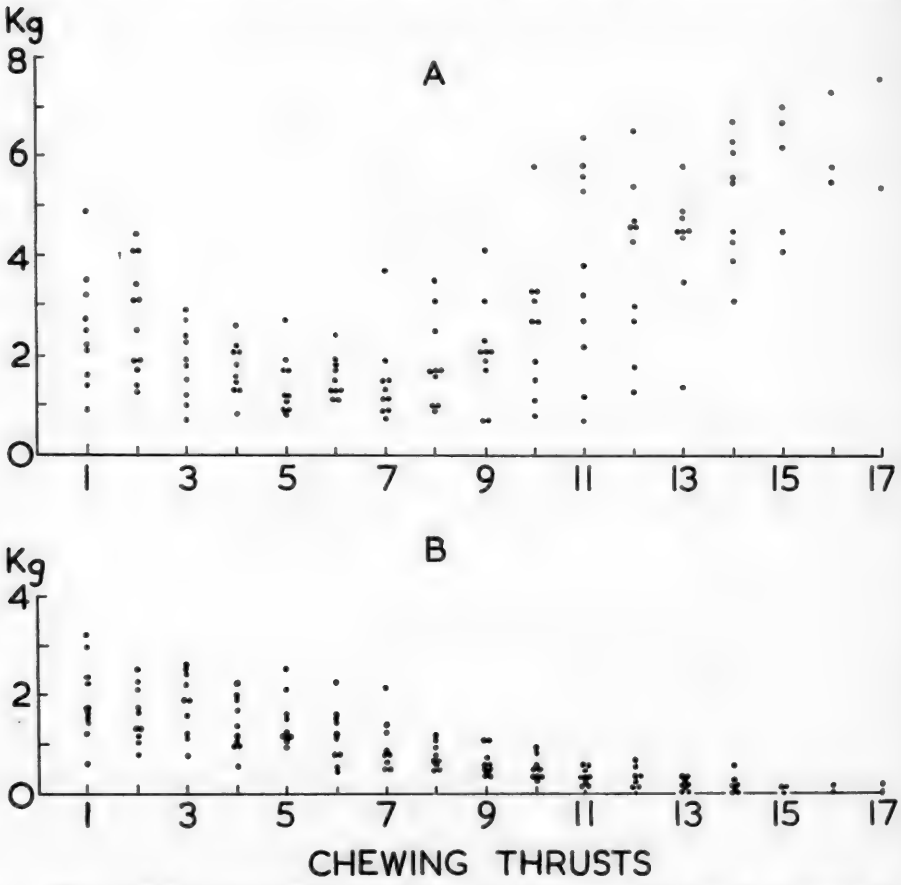

Fig. 4.—Full data from Subject 3 showing the strain-gauge unit loads in kilograms per chewing thrust when chewing with the apparatus in normal occlusion (*A*) and when chewing with the apparatus out of occlusion by 0.5 mm. (*B*).

them by the intervening layer of food. This arrangement simulated the relationship of the teeth during chewing if tooth-to-tooth contact did not occur either through habit or abnormal position of the teeth. From this series it is clear that soft food allows very little force to be transmitted through the teeth and the results add further weight to the view that centric occlusal contact is reached during chewing. All these results were obtained from first molar teeth and according to Yurkstas and Manly,[3] who made measurements of occlusal contact area, this

tooth accounts for approximately 37 per cent of the total for one side of the mouth, or about 18 per cent of the contact area of the whole arch. If the conclusion from the first group of experiments reported here is that the load distribution is uniform over the whole area of contact, then we would expect the raised-bite loads to be approximately 6 times the normal, assuming no reduction in muscular effort. In fact, they are, in 3 subjects of the series, not more than twice the normal, and in one subject about 75 per cent of the normal. This clearly indicates the intervention of some force-regulating mechanism. In none of the subjects was there any conscious limitation of effort when chewing on the raised tooth, although Subjects 2 and 4 reported discomfort at the beginning of the chewing sequences on several occasions.

The loads borne by the teeth are determined by the muscular effort which is, in turn, controlled by the central nervous system. Information on which this control is based must come from pressure receptors in the supporting tissues of the teeth, tension receptors in the muscles of mastication, and receptors in the temporomandibular joint. In addition, it is common experience that pain from the teeth or their adjacent tissues will also contribute to the modification of masticatory effort.

Malocclusion, such as these experiments have attempted to produce, will provide an abnormal pattern of information and it is therefore not surprising that the increase in loads expected from a purely mechanical system has not been recorded. It is reasonable to suppose that the limitation on vertical loads shown by these subjects would also operate on lateral forces which may be potentially more damaging to the periodontal tissues.

SUMMARY

1. Masticatory loads recorded on a lower first molar tooth in 4 subjects during unilateral chewing of bread show a great similarity between those on the chewing side and those on the empty side of the mouth.

2. With the recording apparatus and tooth surface artificially raised above the general occlusal level by about 0.5 mm., the loads are at the most twice the normal. This is much less than would be expected if no force-regulating mechanism were operating.

3. With the recording apparatus lowered by about 0.5 mm., the pattern and magnitude of the loads differs markedly from normal.

The authors wish to thank the subjects for their cooperation and the Nuffield Foundation for a grant which has supported this work.

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THE RELATION OF FLUORIDE IN THE DRINKING WATER TO THE DISTRIBUTION OF FLUORIDE IN ENAMEL

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NUMEROUS studies on the caries-reducing effect of fluorides point to drinking water as being the most reliable vehicle for fluoride supplementation.[1] It therefore becomes of interest to examine the manner by which the concentration of fluoride in the drinking water affects the fluoride content of the enamel.

It is evident from the studies done in this field up to 1951 that an increase in the concentration of fluoride in the water supply results in an increase in the fluoride content of enamel.[2] In these studies which showed a comparatively small difference between the fluoride content from high compared to low fluoride areas, powdered whole enamel was used for analyses. Using another approach, Jenkins and Speirs[3] compared the fluoride content of the surface to that of the subsurface enamel. These workers etched off the outermost enamel with acetic acid and determined the fluoride content in the dissolved enamel and in the residual bulk enamel. By this method more than twice as much fluoride was found in the surface enamel of fluorosed teeth than in the surface enamel of teeth from low fluoride areas. Jenkins and Speirs also concluded that all the fluoride of the enamel was acquired pre-eruptively and that the fluoride content does not vary with age. However, the use of a sampling procedure of grinding rather than etching has shown that posteruptive uptake of fluoride does take place.[4] Therefore, it is desirable to take age into consideration when comparing the fluoride content of teeth from different areas.

The purpose of the present study was to obtain more information concerning the pre-eruptive and posteruptive acquisition of fluoride by enamel. By using the sampling method of Brudevold and co-workers[4] and by working with a number of carefully selected enamel samples, it was hoped that the effect of varying levels of fluoride in the water supply and of age on the distribution of fluoride in enamel would be clarified.

METHOD

A large number of deciduous teeth and of unerupted and erupted permanent teeth were collected by exodontists in Buffalo, New York, Chicago, Illinois, McKinney, Texas, and Post, Texas where the water supply contains 0.1, 0.1, 1.0,

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and 5.0 ppm fluoride, respectively. The erupted permanent teeth were divided according to age into the groups: under 20, 30 to 49, and over 50 years of age. Care was taken to include only teeth from persons who were born and had been living continually in these cities.

Eleven groups of teeth were used. A breakdown of the distribution of tooth types, the distribution of sound and carious teeth and the number of teeth used in each group is tabulated in Table I. Samples of 6 successive layers of enamel

TABLE I
TYPES OF TEETH AND NUMBER OF SOUND (S) AND CARIOUS (C) TEETH IN GROUPS STUDIED

GROUP	ANTERIORS		PREMOLARS		MOLARS		TOTAL NUMBER
	S	C	S	C	S	C	
Deciduous (Chicago, Ill.)	2	–	–	–	1	25	28
Unerupted (Chicago, Ill.)	–	–	2	–	31	–	33
Unerupted (Post, Tex.)	–	–	–	–	6	–	6
< 20 yrs. (Chicago, Ill.)	–	5	13	2	–	3	23
< 20 yrs. (Buffalo, N. Y.)	1	–	–	13	–	37	51
< 20 yrs. (McKinney, Tex.)	2	–	4	1	–	1	8
< 20 yrs. (Post, Tex.)	5	2	4	4	4	8	27
30-49 yrs. (Post, Tex.)	12	2	9	1	5	7	36
> 50 yrs. (Chicago, Ill.)	8	19	1	5	2	6	41
> 50 yrs. (Buffalo, N. Y.)	11	20	1	8	1	24	65
> 50 yrs. (McKinney, Tex.)	2	1	5	11	10	16	45

from the teeth in each group were obtained by grinding with a diamond stone according to a method previously described.[4] Because of the irregularity of the occlusal surface and the question of attrition, the occlusal and incisal surfaces were not used. It was estimated that the sum of the 6 layers ground from any tooth represented approximately half its total enamel thickness.

The weight of the sample obtained per layer in each group is given in Table II. An index of thickness for each layer was obtained by dividing the weight of enamel ground per layer by an estimated index of the total surface area ("surface score") of the intact enamel surfaces from which the enamel was ground.[4] The total "surface score" for each group of teeth is given at the bottom of Table II and the "thickness score" of each layer is tabulated in Table III.

The enamel was ashed overnight in platinum dishes at 575° C. and the fluoride separated by the distillation method of Willard and Winter[5]; fluoride in the distillate was determined by the titration method of Smith and Gardner.[6]

RESULTS

The fluoride concentration in the enamel from the surface inward is given in Table IV for all the groups studied and is plotted in Fig. 1 for 7 of these groups. The 7 groups include under 20 and over 50 age groups from cities with 0.1 ppm or 1.0 ppm fluoride in the water supply, as well as the unerupted, under 20, and 30 to 49 age groups from a city with 5.0 ppm fluoride in the water supply.

The data show that in all groups there is a much higher concentration of fluoride in the enamel surface than in the inner enamel. A comparison of the over 50 with the under 20 age groups reveals that at levels of 0.1 and 1.0 ppm

TABLE II
WEIGHTS IN MILLIGRAMS OF GROUND SAMPLES OF SUCCESSIVE LAYERS OF ENAMEL

LAYER	DECIDUOUS CHICAGO, ILL.	UNERUPTED CHICAGO, ILL.	UNERUPTED POST, TEX.	< 20 YRS. BUFFALO, N. Y.	< 20 YRS. CHICAGO, ILL.	< 20 YRS. MC KINNEY, TEX.	< 20 YRS. POST, TEX.	30-49 YRS. POST, TEX.	> 50 YRS. BUFFALO, N. Y.	> 50 YRS. CHICAGO, ILL.	> 50 YRS. MC KINNEY, TEX.
1	434	410	85	465	233	70	287	318	252	236	375
2	316	380	59	411	183	55	311	361	226	206	320
3	340	370	51	465	188	63	277	268	201	218	323
4	349	364	59	415	186	61	274	348	187	234	378
5	313	325	62	375	163	53	270	239	228	250	362
6	371	370	74	423	210	57	270	286	250	248	450
				Surface Score in the Different Groups of Teeth							
Surface score	49	97	17	84	37	12	53	61	45	65	85

TABLE III
THICKNESS INDEX OF LAYERS GROUND FROM THE DIFFERENT GROUPS OF TEETH

LAYER	DECIDUOUS CHICAGO, ILL.	UNERUPTED CHICAGO, ILL.	UNERUPTED POST, TEX.	< 20 YRS. BUFFALO, N. Y.	< 20 YRS. CHICAGO, ILL.	< 20 YRS. MC KINNEY, TEX.	< 20 YRS. POST, TEX.	30-49 YRS. POST, TEX.	> 50 YRS. BUFFALO, N. Y.	> 50 YRS. CHICAGO, ILL.	> 50 YRS. MC KINNEY, TEX.
1	8.8	4.2	4.9	5.5	6.3	5.8	5.4	5.2	5.6	3.6	4.4
2	6.4	3.9	3.4	4.9	4.9	4.6	5.9	5.9	5.0	3.2	3.8
3	6.9	3.8	3.0	5.5	5.0	5.2	5.2	4.4	4.5	3.4	3.8
4	7.1	3.7	3.4	4.9	5.0	5.0	5.2	5.7	4.2	3.6	4.4
5	6.4	3.4	3.6	4.5	4.4	4.4	5.1	3.9	5.1	3.8	4.3
6	7.6	3.8	4.4	5.0	5.7	4.8	5.1	4.7	5.6	3.8	5.3

TABLE IV
PARTS PER MILLION OF FLORIDE IN SUCCESSIVE LAYERS OF ASHED ENAMEL FROM COMMUNITIES WITH 1.0 AND 5.0 PPM FLUORIDE IN THE WATER SUPPLY

LAYER	DECIDUOUS 0.1 PPM F CHICAGO, ILL.	UNERUPTED 0.1 PPM F CHICAGO, ILL.	UNERUPTED 5.0 PPM F POST, TEX.	< 20 YRS. 0.1 PPM F BUFFALO, N. Y.	< 20 YRS. 0.1 PPM F CHICAGO, ILL.	< 20 YRS. 1.0 PPM F MC KINNEY, TEX.	< 20 YRS. 5.0 PPM F POST, TEX.	30-49 YRS. 5.0 PPM F POST, TEX.	> 50 YRS. 0.1 PPM F BUFFALO, N. Y.	> 50 YRS. 0.1 PPM F CHICAGO, ILL.	> 50 YRS. 1.0 PPM F MC KINNEY, TEX.
1	240	616	3,190	499	460	889	3,370	3,350	971	1,080	1,552
2	129	257	2,120	162	114	363	1,710	2,060	512	540	727
3	55	143	1,580	108	93	255	1,124	1,440	320	305	328
4	54	100	1,133	63	–	171	926	1,057	297	190	227
5	49	70	875	76	51	133	811	1,087	194	198	219
6	50	52	793	42	–	129	570	858	150	114	158

Layer 1 is the outermost layer of enamel; layer 6 is the innermost.

fluoride in the water supply there is an increase in the fluoride concentration of the outer as well as the inner enamel with age. The magnitude of this increase in the outer enamel is considerably greater than that in the inner enamel.

The fluoride values obtained for mottled enamel from the 3 groups of teeth from a 5.0 ppm fluoride area are particularly interesting. Contrary to the findings in the other groups there was an increase in fluoride with age only in the inner enamel while the amount of fluoride in the surface enamel remained unchanged.

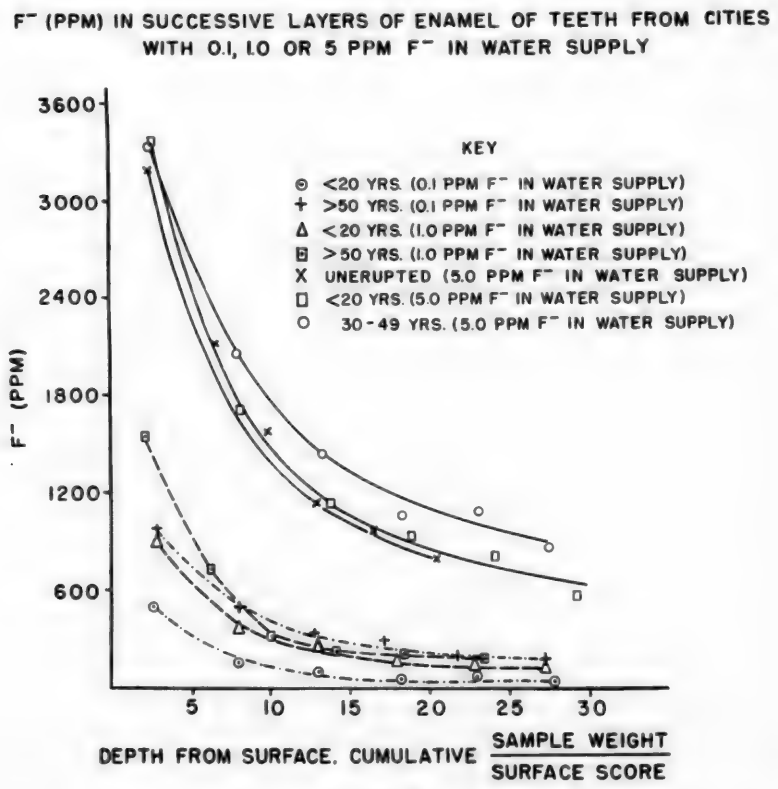

Fig. 1.

The results also show that an increase in the fluoride content of the water supply results in an increase in the fluoride content of enamel. For example, mottled enamel was found to have a very much higher fluoride concentration throughout than the enamel from all the other groups. Moreover, fluoride contents of the outer and inner enamel of the under 20 age group from the 1.0 ppm area are considerably greater than those of an equivalent age group from a 0.1 ppm area. Although significantly greater amounts of fluoride are present in the enamel surface of the over 50 age group from the 1.0 ppm area compared to that of the same age group from a 0.1 ppm area, there is little difference in the fluoride contents of the inner enamel of these 2 groups.

From Table IV, it is evident that the 2 under 20 age groups, from cities with 0.1 ppm fluoride in the water supply, show similar fluoride concentrations in the enamel. This is interesting in view of the fact that 98 per cent of the teeth in the 1.0 ppm group were carious in comparison with 43 per cent in the 0.1 ppm group.

DISCUSSION

Our findings agree with those of Jenkins and Speirs[3] insofar as they show that an increase in the fluoride content of the water supply results in an increase in the fluoride content of the enamel. However, contrary to their findings,[3] our results confirm previous findings in this laboratory that there is a posteruptive uptake of fluoride as evidenced by the increase in the fluoride content of the enamel with age even when the water supply contains only 0.1 ppm fluoride.[4]

It has been suggested[4] that fluoride deposition in enamel takes place in 3 stages, viz., (1) during the period of enamel formation, when fluoride is deposited in the enamel matrix, (2) after calcification is complete but before eruption, when more fluoride is taken up by the external surfaces of enamel from the tissue fluids, and (3) after eruption and throughout the life span of the teeth, when fluoride from the drinking water, food, and saliva is taken up by the enamel surface.

This premise is confirmed by our findings. Therefore, in relating the fluoride content of the water supply to the fluoride content of enamel it becomes necessary to distinguish between the fluoride that is deposited during tooth formation and pre-eruptively and the uptake of fluoride posteruptively.

From the data it is clear that when the fluoride content of the water supply is increased from 0.1 ppm to 5.0 ppm there is a considerable increase in the amount of fluoride that is deposited in the enamel during tooth formation. It is unfortunate that no unerupted teeth were available from a 1.0 ppm fluoride area, but the results reported here and previous findings[4] show that the inner enamel from unerupted teeth and erupted teeth from persons under 20 years of age from equivalent fluoride areas have a similar fluoride content. The fluoride concentrations of the inner enamel of the under 20 years age groups from 0.1 and 1.0 ppm fluoride areas suggest that, at a level of 1.0 ppm, there is a slight increase in the deposition of fluoride during tooth formation. It has been found that an increase from 0.06 to 1.12 ppm in the concentration of fluoride in the water supply results in an increase from 0.014 to 0.040 ppm in the fluoride content of the blood.[7] Since the amount of fluoride deposited in the enamel pre-eruptively is dependent on the fluoride level in the blood, it is conceivable that such an increase in the fluoride level of the drinking water would result in a slight increase in the deposition of fluoride during tooth formation.

At the levels of 0.1 and 1.0 ppm fluoride in the water supply, the post-eruptive uptake of fluoride is particularly apparent in the outermost portion of the enamel. This suggests that the posteruptive acquisition of fluoride by the enamel is due to direct contact of the drinking water resulting in an accumulation

of fluoride in the surface enamel. It is interesting to note that it takes almost a lifetime to increase the fluoride content of the surface by 600 ppm. It is also interesting that the fluoride content of the enamel from an under 20 years age group from a 1.0 ppm fluoride area is almost equivalent to that of an over 50 years age group from a 0.1 ppm area.

At the level of 5.0 ppm fluoride in the water supply, the posteruptive uptake of fluoride is only apparent in the inner enamel. This suggests that the concentration of about 3,000 ppm fluoride found in the surface enamel of unerupted teeth from this area represents a level of fluoride saturation so that further uptake of fluoride occurs chiefly in the yet unsaturated inner enamel. This would be facilitated by penetration through the mottled enamel due to deficiencies in the interprismatic substance.

Several studies[8-10] showed a marked reduction in the DMF rate when the fluoride content of the water was increased from 0.1 ppm or less, to 1.0 ppm. Our results show a difference of over 300 ppm fluoride in the external enamel from such areas in each age group. Since carious lesions begin at the enamel surface it is very likely that the caries-reducing effect of water fluoridation at the level of 1 ppm is due mostly to the resultant increase in the fluoride content of the enamel surface. This is in accord with the concept of Armstrong and Brekus that there is a direct relationship between the fluoride content of enamel and caries susceptibility.[11] However, since many factors, in addition to fluoride, influence the carious process, the fluoride-caries relationship is not a simple one and teeth with the same fluoride content do not necessarily show the same incidence of dental caries. This is clearly demonstrated by our finding that there were no differences in the fluoride contents of sound enamel from carious and noncarious teeth from equivalent fluoride areas. Similar conclusions were reached by McClure and Likins,[2] and by Harrison.[13]

It is interesting that the surface enamel of normal teeth has an equivalent or higher fluoride content than the subsurface enamel of mottled teeth. This suggests that mottling is not caused by the actual amounts of fluoride deposited in the enamel but rather by an increase in the amount of fluorine ion present during enamel formation. It is unfortunate that there is no agreement in the literature on the amount of fluoride normally present in the blood. However, since a fluoride concentration as low as 2 ppm in the water supply can produce mottled enamel it is apparent that only a slight increase in the fluoride content of the blood is enough to affect the function of the ameloblasts. Enamel matrix formation begins at the dentinoenamel junction and proceeds outward. If the toxic effect of fluoride on the ameloblasts were most marked by the time the last portion of the enamel matrix was laid down, the histologic deficiencies in the interprismatic substance of mottled enamel would be most marked in the outer third.

SUMMARY

1. A comparison was made of the fluoride content of successive layers of enamel from groups of deciduous teeth, unerupted permanent teeth, and erupted

permanent teeth of different age groups. The teeth were collected from persons who were born and had lived continually in cities where the water supply contains 0.1, 1.0, or 5.0 ppm fluoride.

2. In all groups, a higher fluoride concentration was found in the enamel surface than in the inner enamel layers.

3. At levels of 0.1 and 1.0 ppm fluoride in the water supply, there was an increase in the fluoride content of the outer as well as the inner enamel with age while at 5.0 ppm the increase in fluoride with age was only evident in the inner enamel.

4. An increase in the fluoride content of the water supply results in an increase both in the amount of fluoride deposited in the enamel during tooth formation and in that acquired posteruptively.

The cooperation of 20 dentists who collected the teeth used in this study is much appreciated.

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FLUORIDE PLACENTAL TRANSFER IN THE RAT

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THE often-discussed question of the placental transfer of fluorides on one hand, and the possibility of an existing barrier to fluoride on the other, induced us to continue our interest in this problem. Previous work in these laboratories[1] has indicated that placental fluoride transfer in the rat takes place only at high and unphysiologic fluoride levels. Such studies as these are of nutritional interest, however, in order to learn more about the crystal structure of the tooth of the rat which calcifies in the presence of a high plasma-fluoride level.

Gardner, Smith, Hodge, Overton, and Feltman[2] found that the placental tissue contained a higher concentration of fluoride if obtained from women receiving a communal water supply at 1.0 ppm F than if obtained from women receiving an essentially fluoride-free communal water. The placental tissue also had a higher fluoride concentration than the respective blood plasma. Since excessive fluoride may be toxic to the fetus, this organ may be acting as a barrier to prevent more than trace amounts of fluoride from reaching the fetus. The authors did not determine quantitative estimates of how much fluoride passes from the placenta to the fetus.

Held[3] compared the fluoride in the mothers' and fetuses' blood and found that medications containing fluorides quickly increase the fluoride level of the fetal blood and concluded that no placental barrier exists. Feltman and Kossel[4] found a marked difference in the fluoride content of the cord-blood in a control group and in groups who received CaF_2 in the form of tablets or who used artificially fluoridated water, but only a small difference in the placental tissues. Light[5] did not find a parallelism between placental fluoride values in human beings and the consumption of fluoridated water, but the placental fluoride values, which showed a wide variation, seemed to parallel the milk intake. Truhaut[6] and Roholm[7] felt that a fluoride placental transfer in the rat is possible only after administration of high amounts of fluoride. Knouff, Edwards, Preston, and Kitchin[8] reported a fluoride placental transfer in dogs receiving fluoridated drinking water at a concentration of 25 ppm fluoride or a dose of 50 mg. daily, but could not find an increase in the placental tissue after giving the dogs fluoridated drinking water at a concentration of 5 ppm.

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Schubert[9] reported that the teeth of 8- and 12-day-old pups of mothers receiving 0.1 mg. fluoride as NaF, during pregnancy and lactation, were better developed and mineralized than those of the control group but that at 3 days there was no difference.

Mottled enamel does occur in human deciduous teeth, so it does seem physiologically possible for fluoride to pass the placenta. It remains to be determined what levels must be received and what nutritional states affect its transfer.

EXPERIMENTAL

In this study, 14 pairs of adult rats were used for continuous breeding. Six groups received varying fluoride concentrations in the drinking water at a level of 1, 3, 5, 10, 25, and 50 ppm fluoride as sodium fluoride, respectively. A control group received redistilled fluorine-free water. All groups received the same stock corn diet containing approximately 1.0 μg fluoride per gram.

Following delivery, 2 pups of each litter were sacrificed at birth, 2 at 10 days, and 2 at 30 days of age. The whole carcasses were ashed by methods previously described[10] in order to determine the fluoride skeletal retention as a result of placental transfer.

RESULTS AND DISCUSSION

Table I indicates the number of litters born, the total number of pups from these litters, and the number of rats surviving at each fluoride concentration used in this study. While the higher levels of fluoride do not seem to affect reproduction, there appears to be a tendency for fewer pups surviving as the fluoride concentration is increased.

TABLE I
THE REPRODUCTION RECORD OF RATS RECEIVING DIFFERENT CONCENTRATIONS OF SODIUM FLUORIDE IN THE DRINKING WATER

F CONC. IN DRINKING WATER (PPM)	NO. OF LITTERS BORN	NO. OF RATS BORN	NO. OF RATS SURVIVING	NO. OF SURVIVING PUPS PER LITTER
0	5	30	20	4.0
1	5	33	19	3.8
3	3	13	10	3.3
5	3	13	9	3.0
10	4	14	13	3.3
25	7	30	22	3.2
50	7	35	25	3.6

Table II shows the effect of skeletal fluoride retention from the various concentrations of fluoride used in this study as a function of age of the pups. The data obtained from the animals sacrificed at birth indicate that the mother must receive about 10 ppm F before any appreciable increase in carcass fluoride is found in the pups. At higher levels one finds a clear increase of fluoride storage in the pups with increasing fluoride concentration in the drinking water. The total fluoride in the carcass shows similar data. The fluoride in the pups whose mothers received 0 to 5 ppm fluoride in the drinking water

TABLE II
FLUORIDE STORAGE IN 1-, 10-, AND 30-DAY-OLD PUPS OF MOTHERS RECEIVING VARYING CONCENTRATIONS OF FLUORIDE IN DRINKING WATER

F CONC. IN DRINKING WATER	F IN ASHED CARCASSES OF PUPS					
	1 DAY		10 DAYS		30 DAYS	
PPM	PPM	μG	PPM	μG	PPM	μG
Control	53 ± 9	4.7 ± 0.8	48 ± 7	18 ± 2	–	–
1	50 ± 8	4.6 ± 0.6	50 ± 4	18 ± 1	51 ± 6	106 ± 10
3	51 ± 5	5.5 ± 0.5	58 ± 17	31 ± 9	–	–
5	45 ± 9	5.2 ± 0.8	–	–	–	–
10	77 ± 6	8.6 ± 1.4	50 ± 15	35 ± 13	–	–
25	81 ± 7	8.7 ± 1.0	–	–	–	–
50	115 ± 14	11.9 ± 1.5	102 ± 8	72 ± 8	922 ± 88	2250 ± 200

is about 5 μg, while the fluoride in the pups of mothers receiving 50 ppm fluoride in the drinking water is 12 μg. Thus, while the fluoride in the drinking water is about 20 to 50 times higher, the fluoride storage in the pups is only 2 to 3 times higher.

Similar results were found by analyzing the animals sacrificed 10 days after birth. The fluoride storage in the pups of mothers receiving the low fluoride levels is about 18 μg in the carcass, while the fluoride storage in the pups of mothers receiving 50 ppm fluoride is about 70 μg or about 4 times as much fluoride as in the group receiving the low fluoride concentration. The total fluoride in the carcass at the 10-day period indicates a pronounced skeletal increase when compared to those values obtained from the animals sacrificed at birth. This finding suggests a mammary gland transfer and needs further investigation. However, it may also be a result of external fluoride contamination, although this does not seem to be the correct interpretation since pups at this age do not routinely eat or drink from the food and water provided.

Due to sacrificing the pups for analysis at birth and at 10 days of age and since the litters in general were small, there were only a few animals remaining for analysis at 30 days. Here the increase in the fluoride storage is high and corresponds approximately with the increased fluoride concentration in the water. In this case, the fluoride is retained as a function of fluoride ingestion.

These results show that a fluoride placental transfer is possible, and also suggest that some of the fluoride may transfer from the mammary glands, especially at the higher levels. In general, it is considered that the fluorine in milk is not absorbed to any appreciable degree unless the concentration exceeds 10 μg F/ml.[11, 12] and thus will not contribute significant amounts of fluorine to the fetus if present at low concentrations. It appears that only a small percentage of the offered fluoride passes the placental tissue. This is more evident in the fluoride concentration of ashed carcasses. The percentage of fluoride storage of the 1-, 10-, and 30-day-old pups in each group is approximately the same. But in the 30-day-old pups where mothers received 50 ppm F, the fluoride concentration of the carcass is approximately 9 times higher than in the corresponding 1- and 10-day-old pups.

These data are of interest, of course, but data obtained in rats cannot be used unequivocally to interpret human data. The importance of these studies rests primarily with our continued interest to determine if fluoride can be incorporated into the rat molars during calcification and to determine what dietary factors affect the rate of deposition. These investigations can be useful in considering what levels of fluoride can safely be ingested without fear of placental transfer and, thus, may be helpful not only from a toxicological investigation, but in an evaluation of such factors as caries resistance in the deciduous dentition of the human being.

SUMMARY

A total of 7 groups of rats received different amounts of fluoride in the drinking water during pregnancy and lactation. The skeletal fluoride storage of the pups was determined. These data indicate that drinking water at a concentration of 10 ppm F must be received by the mother before any appreciable increase in carcass fluoride is found in the pups. Data are presented which indicate a mammary gland transfer, especially at higher fluoride levels.

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THE ROLE OF PSYCHOLOGIC PREDISPOSITION IN DENTAL CAVITY FORMATION

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IT is generally agreed that some relationship exists between psychologic factors and general bodily disorders. Furthermore, in recent years, a growing quantity of statistically evaluated research data has been presented to substantiate the fact that oral pathologic conditions could have psychologic bases.[3, 4, 15-20, 22]

The largest number of studies presenting these oral psychosomatic data have adhered to the medically orthodox and psychologically spectacular belief that if dental-psychologic relationships do exist, the resultant pathology can only be precipitated by physiologic mechanisms; these mechanisms being dependent upon, or resultant from, neurotic conditions within the organism.

It was suggested, however, by Jones and Manhold,[5] that psychosomatic manifestations may result not from mechanisms which are in accord with the commonly accepted conversion-from-neurosis theory but rather from simple neglect; this neglect possibly being dependent upon facets of personality which lead to deleterious habit formation. Furthermore, results of investigation of this suggestion[19] demonstrated its apparent validity; that is, they found, using the California F scale, that (1) persons who hold nonauthoritarian attitudes differ markedly in background, aptitudes, and interests from those who are more authoritarian, and (2) that these persons tend not only to neglect their physical health in general, but also tend to have a higher DMF rating, i.e., a greater number of dental cavities than do their more authoritarian peers.

These results, together with the afore-mentioned findings supporting the dysfunctional postulate, caused the recent formulation of a dichotomous hypothesis for psychosomatic disease processes.[20]

The present study presents (1) an attempt to delineate more clearly factors of personality associated with the formation of dental caries through neglect, and (2) an attempt to test further the dichotomous psychosomatic postulate.

Subjects.—The subjects employed in this study were Naval Aviation Cadets of the Naval Air Station, Pensacola, Florida, and Naval Retrainees from Portsmouth Naval Retraining Center, Portsmouth, New Hampshire.

The cadets are a fairly homogeneous group from the standpoint of education and socioeconomic background, and range from 18 to 27 years of age.

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The retrainees are a somewhat less homogeneous group but also have much in common in a much lower educational level and a generally lower socioeconomic foundation. Their age range is from 17 to 50, with a mean approximately 2 years older than that of the cadets.

System of Measurement.—The systems of measurements employed in this study were a DMF Surface Index[2] of dental caries and the Pensacola Z Scale.[6, 7]

The Pensacola Z Scale is a new psychologic instrument which purports to measure attitudes of personal autonomy and heteronomy. These attitudes are similar to the authoritarian-nonauthoritarian attitudes measured by the California F Scale, but are referred to by the test author as more pure measures of a type of personality ("a terminal measure of personal autonomy"),[6] because the scale was specifically constructed by a group of preparatory studies[1, 8-14] to parcel out the sociopolitical aspects existing in the authoritarian-nonauthoritarian syndromes.

METHODS

The Z Scale, after construction and validation against the California F Scale on the Naval Cadet population, was administered to 311 Naval Retrainees at Portsmouth Naval Retraining Center. Each man was examined clinically and radiographically so that his dental condition could be evaluated and a DMF Surface Index rating ascertained. The results of these examinations were punched on IBM cards and submitted to statistical analyses.

RESULTS

The results obtained from these analyses are presented in Tables I through IV.

The mean Z Score in the Cadet population is relatively stable (Table I). No significant difference exists between the two samples. (From correspondence with the author of the Z Scale, it has been learned that further samples have produced mean and sigma values which have varied little from these figures.) The figures obtained for the total population from the values for Samples 1 and 2 are also shown.*

Table II presents the comparison of means and standard deviations for the groups who took the Z test for this study. The population of 311 Naval Retrainees was found to have a mean score of 38.85 with a standard deviation of 5.74. These values, when compared with the Cadet mean of 35.45 and standard deviation of 6.71 show a critical ratio of 8.94. The difference between the two means is significant beyond the 0.001 level of confidence.

*The total population values were obtained by substitution in the formulas:

$$M = \frac{(N_1)\ (M_1) - (N_2)\ (M_2)}{N_1 - N_2}$$

and

$$\sigma = \sqrt{\frac{(N_1)\ (\sigma_1)^2 + (N_2)\ (\sigma_2)^2}{N_1 + N_2} + \frac{(N_1)\ (N_2)\ (M_2 - M_1)^2}{(N_1 + N_2)^2}}$$

TABLE I

MEANS AND STANDARD DEVIATIONS FOR THE Z SCALE IN THE NAVAL CADET POPULATION USED IN THIS STUDY

	N	MEAN	S.D.
First sample	766	35.51	6.33
Second sample	311	35.31	6.85
Total population	1,077	35.45	6.71

TABLE II

COMPARISON OF MEANS AND STANDARD DEVIATIONS FOR THE Z SCALE IN THE NAVAL CADET AND RETRAINEE POPULATIONS

	N	MEAN	S.D.	σm	C.R.	SIGNIFICANCE
Cadets	1,077	35.45	6.71	0.204	8.94	0.001
Retrainees	311	38.85	5.74	0.322		

Table III presents a comparison of the mean and standard deviation values of the DMF Index Scores of Naval Cadet and Retrainee populations. The population of 311 Naval Retrainees were found to have a mean DMF score of 24.49, with a standard deviation of 16.46. These values, when compared with the Cadet population mean of 27.22 and sigma of 15.55, show a critical ratio of 2.60, a result significant at the 0.01 level of confidence.

The results presented in these tables, therefore, may be interpreted to mean that a significant difference exists between the Naval Cadet and Naval Retrainee populations with respect to both personality type and dental caries.

TABLE III

COMPARISON OF MEANS AND STANDARD DEVIATIONS OF THE DMF INDEX SCORES IN THE NAVAL CADET AND RETRAINEE POPULATIONS

	N	MEAN DMF SCORE	S.D.	σm	C.R.	SIGNIFICANCE
Cadets	1,019	27.22	15.55	0.487	2.60	0.01
Retrainees	311	24.49	16.46	0.935		

Table IV presents the intercorrelational value obtained between DMF and Z Scale scores in the Retrainee population. As may be seen from this table, a correlation of 0.11, significant at the 0.05 level of confidence, was obtained between the DMF and the Z Score in this population of 311 subjects.

This result could be interpreted to mean that greater tendencies toward heteronomy (and the factors which compose this syndrome) are related to a higher incidence of dental caries experience.

TABLE IV

CORRELATION AND SIGNIFICANCE VALUES BETWEEN DMF SCORES AND THE Z SCALE IN THE NAVAL RETRAINEE POPULATION

DMF-Z CORRELATION COEFFICIENT	N	0.05 LEVEL OF CONFIDENCE	SIGNIFICANCE OF RESULTS
0.11	311	0.11	0.05 level

DISCUSSION AND CONCLUSIONS

The results of this study present some interesting relevancies to the psychosomatic disease hypothesis, and especially in regard to application of this postulate to the specific discipline of dental pathology. It is true that, from a very literal standpoint, we may safely say only that a definite difference exists between the Cadet and Retrainee populations with respect to both their dental health and the facets of personality measured. However, in the light of the fact that the Pensacola Z Scale (1) is by definition "a terminal test of personal autonomy," (2) was constructed on results of research with the California F Scale, and (3) enjoys relatively high correlation with the F Scale, we may hypothesize that the factor of psychologic disposition, or habit of neglect, existent in the cadet or more autonomous person could be the factor leading to the higher dental caries rating. Similarly, the habit of physical care exhibited by the heteronomon could be the factor giving him a lower caries incidence. The results have also demonstrated, however, that the stronger are the individual's tendencies toward heteronomy, i.e., the more anxious, rigid, and hostile he is, the higher his dental caries rating appears to be.

Thus, from the results of this study, it would appear that we could conclude that (1) factors of personality associated with the formation of dental caries have been more clearly delineated, and (2) further support has been offered for the dual concept of psychosomatic affection.

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Erratum

In the article by Dr. Stanley M. Garn and Dr. Arthur B. Lewis entitled "Relationship Between the Sequence of Calcification and the Sequence of Eruption of the Mandibular Molar and Premolar Teeth," which appeared in the December issue of the JOURNAL, the second paragraph on page 995 should read as follows:

> On the other hand, assigning the M_2P_2 sequence to Sinanthropus, as Weidenreich[7] did, appears to be an outright mistake. The deep location of the follicle of P_2, as contrasted with the more superficial location of the germ of M_2, is by no means unusual. Judging from the radiographs published by Weidenreich,[8] Sinanthropus (B_1) was P_2M_2 or (P_2M_2) in formation, definitely not M_2P_2 and, therefore, likely to be P_2M_2 in eruption, barring the chance of a reversal as noted above.[9, 10, 11]

THE CLINICAL DENTAL TRIAL

I. A New Caries-Inhibitory Agent

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VARIOUS methods of analyzing the clinical effectiveness of caries-inhibitory agents were presented in an earlier published paper.[1] Since then, a conference dealing, in part, with the same general theme was held and a report of the discussions published.[2] Of particular interest is the part written under the leadership of A. L. Russell, who also summarized the over-all report.[3] Other papers dealing with broader aspects of the subject of clinical trials have been published.[4, 5, 6] In addition, a relatively large number of papers have appeared which purport to show the effectiveness of new caries-inhibitory agents, particularly of dentifrices. Believing that these studies are a manifestation of the increasing interest in the clinical dental trial, the authors would like, in this present paper, to discuss, in greater detail, concepts of the design and analysis of studies dealing with caries-inhibitory agents, particularly with dentifrices.

SETTING UP THE STUDY

Preliminary Testing.—When a new agent is found which gives promise of being an effective caries-inhibitory agent, exhaustive in vitro tests should be performed to determine its chemical and physical as well as its pharmaceutical properties.

If the prospective caries-inhibitory agent passes this screening, it should be subjected to in vivo animal studies to determine its effectiveness in the oral environment as well as a careful evaluation of its toxicity (including the effect of prolonged usage, i.e., immediate and latent effects), and a determination of its effect on the indigenous flora and on the other organ systems.

The next step is the testing of the agent in a pilot study on human beings. The size of the usual samples (experimental and control groups) seems to run between 25 and 100 individuals. These samples are often selected from those individuals who are readily accessible to the investigator and who can be studied in detail for items other than caries inhibition alone. Auxiliary tests as outlined above and discussed by Hein should be performed at this time.[7]

If the prospective agent has passed these three preliminary screenings, the next step is the clinical trial. Since a properly run clinical trial is expensive and time consuming, the investigator should be fairly certain that the

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new agent offers great promise, as based upon the preliminary studies, before embarking on a clinical trial. If these preliminary studies point to a rather equivocal effectiveness or, at best, a slight improvement over existing agents, a clinical trial should be viewed rather dimly.

Size of the Group(s).—In setting up the clinical trial, one of the first factors to be considered is the size of the group(s) to be studied. The information obtained from the pilot study will give the investigator an idea of what might be expected in the larger clinical trial. Furthermore, from the pilot study, the investigator has an idea of the variation of the measurements between the individuals in these 2 groups and can use this to estimate the variation that can be expected in a larger study. The authors have published papers dealing with the concepts underlying the estimation of sample size in experiments, as well as describing methods for calculation of the size of these samples using several different experimental designs.[8] One further thing must be kept in mind—in many clinical trials, individuals included at the beginning may not be available for final evaluation for various reasons. Allowances must be made in estimating the samples to take care of this contingency.

Selection of Study Groups.—The selection of groups for study has often been made on the basis of personal contacts or relationships with administrators of schools, of public or private institutions, or even of industrial organizations. While such personal contacts are quite helpful, in fact even desirable, in order to ensure full cooperation of the various administrators having to do with the study population, there are other very important considerations in the selection of these groups.

Since dental caries is most active during childhood and adolescence, the best trial would be run in a group in which these ages were greatly represented, rather than in an older age population in which the carious process is no longer active. While this may seem quite obvious, the first clinical trials of some agents have been run in such older groups. The lowest age group studied should be one in which sufficient permanent teeth are present at the start to give a large enough susceptible tooth population. While deciduous teeth also are attacked by caries, accurate record keeping and final evaluation of results are made extremely difficult due to the normal exfoliation of these teeth in a very variable pattern. Thus, unless a study is specifically set up to evaluate the effectiveness of the caries-inhibitory agent on deciduous teeth, it would be advantageous to eliminate consideration of these teeth from the study. Similarly, while some clinical trials may be pointed for specific problems, the chronological ages which would provide the most suitable "dental ages" for the clinical trial would usually run from 10 or 11 to 19 years of age. While the study need not be confined to these age groups, the distribution of subjects should be concentrated within these age groups.

The population selected for the clinical trial should be a fairly stable one. This stability should be reflected in a relatively low loss of the population in the

groups over the study period, as well as in the consistency of carrying out the desired method of utilization of the agents being compared. Undoubtedly, a "captive" type of population would meet these requirements to a greater extent than would one consisting of individuals who are together only part of the time, i.e., an institutionalized population rather than a public school population. Children attending school as day students can, of course, be used as the groups under study, but the investigator must make every effort to obtain the whole-hearted cooperation of the parents as well as the school administrators, teachers, and children themselves. When this cooperation is not fully obtained, it is not inconceivable that extraneous factors may be introduced which can influence the outcome of the clinical trial. For example, some children might have topical applications of fluoride salts by their private dentists or may use any one of a number of dentifrices now on the market which may or may not have a caries-inhibitory effect, or may not be consistent in their oral hygiene patterns away from the prescribed routine at school. With institutionalized populations these extraneous factors are amenable to much more careful control and such possible influencing factors as dietary intake can also be better controlled or at least recorded. On the other hand, institutionalized populations may not be representative of children of similar ages in the population as a whole and any conclusions drawn from the results of the clinical trial may not apply to the over-all population.

Comparability of Study Groups.—The composition of the groups receiving the test, or experimental agent, and the control agent should be such that they are comparable with respect to those factors (known and unknown) which influence dental caries susceptibilty. Among the known factors are age, sex, race, caries experience, fluoride intake, dietary practices, and, perhaps, socioeconomic status. This usually cannot be accomplished by assigning one dentifrice to one intact group in a particular school and the other dentifrice to an intact group in a different school. While the employment of such intact groups certainly will ease the administrative problems involved in any clinical study, one group may be quite different from the other with respect to the ponderable factors listed above which could produce confusion in interpreting the results obtained from such a study.

While a random assignment of the children available to the study groups would assure comparability within chance limits for the known as well as the unknown factors, it is usually advisable (as well as often simpler) to control some of the known factors by a procedure of stratification. With an institutionalized population, the children may be stratified with respect to age and then the distribution of the dentifrices randomized within these age strata. Stratification by age usually approximately exists in the various grades of school and also effects some sort of stratification for other known factors, such as caries experience. Furthermore, since the domiciles are usually separated by sex, this factor can also be controlled. If race is also to be controlled better than by randomization alone, it can be balanced either by limitation of

the study to one racial group or by specific assignment of the experimental and control agents within racial groups. While not quite so simply done, comparability can also be obtained in noninstitutionalized populations. Dietary factors are less readily controlled in these latter populations, however.

A problem which sometimes arises in clinical trials of this kind is that of switching of the agents being tested as a result of the natural curiosity of the subjects—the desire to find out what the other fellow is using. This may be overcome at least partially by the variety of code numbers assigned to the agents.

In the simplest (and perhaps "standard") case, a dentifrice containing the supposed caries-inhibitory agent is to be compared with one not containing the agent. Both dentifrices should be alike in every respect (chemical composition, color, consistency, flavor, packaging) except that the portion of dentifrice containing the test agent is replaced by inert filler in the control dentifrice. The two dentifrices should differ only by code numbers. Numbers from 1 to, say, 12 can be used to label the dentifrices, with half of them denoting experimental and half control.

The range of numbers used as the code can be increased to make switching of dentifrices even more difficult. On the other hand, a continuous numbering of dentifrices is not a wise procedure administratively, since it is usually necessary to supply additional dentifrice to the subjects. An additional supply can be given without "breaking the code" each time, by simply giving the specifically numbered agent. Thus, by using a random permutation of the numbers from 1 to 12 (obtained from a table of random numbers[9]), half of these code numbers can be assigned to the experimental dentifrice and the other half to the control. One random permutation gives us, for example, 4, 5, 7, 8, 10, 1, 2, 12, 3, 9, 6, 11. Consequently, the code numbers 4, 5, 7, 8, 10 and 1 are assigned to the experimental dentifrices and the remainder to the control. The dentifrices can then be assigned in rotation, from 1 to 12, to the children in each classroom. In the long run, the experimental and control groups will have the same numbers. Furthermore, this method will ensure comparable groups with respect to those factors capable of influencing caries activity which were not already controlled by stratification.

After the initial examinations have been performed and, if possible, even before the clinical trial has commenced, it is wise to check the comparability of the control and experimental groups with respect to the known factors. This is particularly true if intact groups have been used or if the selection of subjects has been through "haphazardization" rather than by randomization. Fortunately, if one has ensured comparability with respect to age by stratification procedures, the 2 groups are likely to be comparable with respect to many other relevant factors, especially if the treatments have been assigned at random within the age groups. Even when this has been done, it is wise to check for comparability, if for no other reason than to silence the critics. The proportion of each group which is male or female, or white or nonwhite,

etc., can be calculated. If any appreciable difference exists, the assessment of chance can be made by the usual test of proportions, using the $\sqrt{\frac{pq}{n}}$ formula for the standard error. If chance cannot easily account for such a difference, doubt may be cast upon the method of allocation of subjects to the experimental and control groups and the validity of the whole clinical trial may be in jeopardy. Unfortunately, lack of significance is not complete assurance that the groups are comparable with respect to the factor in question.

If stratification according to age has been done by the procedure suggested, then the groups should be completely similar with respect to this factor. However, in other cases, the comparability should be checked by calculating the average (mean) age in each group. If they are appreciably dissimilar, the significance of the difference can be tested by comparing the difference between the means with the standard error of the difference between means.

The most important comparison to be made is between the number of teeth available for attack by caries in the experimental and control groups (i.e., originally noncarious teeth at the start of the study). This would be somewhat akin to studying the effect of a preventive agent such as a vaccine against a particular disease in two populations. We must make sure that each population presents a similar number of susceptibles before starting the study. In a clinical caries study, the further fact that each individual contributes teeth in batches to the over-all group necessitates the comparison in terms of the average number of eligible teeth per individual. Fortunately, if the age distributions are similar these averages are also usually similar.

Because clinical caries trials involve children, permanent teeth erupt into the mouth during the study period. We therefore feel it to be advisable to compare not only the distributions of noncarious teeth originally present in the mouth at the start of the trial, for the experimental and control groups, but also the distributions of the teeth erupting during the study period for the 2 groups. A final test of comparability which is not independent of the other 2, would consist of comparing the average number of noncarious permanent teeth in the mouth during the whole study period.

Mention should be made of the use of matched pairs in the selection of the study groups. In this case, stratification is carried on to a much greater extent than that previously suggested. In this procedure, 2 individuals are found who are the same with respect to the known factors affecting the outcome (i.e., caries activity), with one being assigned to the experimental and one to the control group. As Russell and his co-workers have stated[2]:

> In practice this scheme has several drawbacks. It breaks down completely with the emergence of an unanticipated or overlooked factor of consequence for which matching has not been carried out. At best, from one quarter to one third of the children available for study ordinarily are eliminated at the outset because no precisely-

> matching partner is available; and whenever one of the pair does not appear for re-examination at the conclusion of the study his opposite number must be discarded as well. As a consequence a great part of the examination is unproductive. From the statistical standpoint this waste is not justified, since this method shows little gain in precision over the "balanced study."[10]

Examination and Recording Procedures.—In examining the population of the study and control groups to obtain base line data, as well as to obtain data from subsequent examinations, examining and recording errors should be kept to a minimum.[11] Undoubtedly, the more examiners utilized the greater this type of error would tend to be. It would be preferable, therefore, to use as few examiners as feasible, and also to spell out thoroughly to these people the exact criteria to be used in recording carious lesions, missing teeth, etc.[12] In subsequent examinations, the examiners should not record their observations upon the same record card used for any of the previous examinations, but the type of form and method of recording should be the same. The examiner should have no knowledge of the agent (dentifrice) being used by the patient being examined, nor of the results of the previous examinations of the individual in question.

There are many types of record forms available for recording caries status. The forms to be employed in a dental caries clinical trial should be as simple as possible (i.e., without unnecessary items) facilitating filing and tabulation, particularly that involved in comparisons with other examinations on the same individual. Space should be provided for such basic notations as name, residence, age (in terms of last birthday or nearest birthday throughout the study), date and number of the examination, and the code number of the agent being used by the patient. Space should also be provided for noting any factors which may influence the caries activity of the patient, i.e., previous exposure to fluorides, dietary restrictions, etc., as well as space for identification of the examiner. The body of the record form should be devoted to the actual notations of the caries status.

These notations as to the caries status of the patient at the time of examination should be kept as simple as possible. The exact notations depend upon what final comparisons are to be made. If great detail is desired in order to obtain many types of information, the U.S.P.H.S. form used for fluoridation studies is quite applicable. In a trial in which the tooth is the unit assessed, the recording can be made quite simple. Each of the 32 possible tooth spaces should be diagrammed and numbered in some standard manner, i.e., Palmer notation. The examiner should record the status of each tooth space as containing a noncarious tooth or unerupted tooth. Only permanent teeth should be recorded. This type of very simplified recording will show quite readily the number of teeth eligible for attack by dental caries. Subsequent examinations will disclose which teeth have been attacked by caries, and which teeth have erupted into the mouth, and which of the latter have been attacked by caries.

The simplified method of recording caries status suggested above is based upon the authors' belief that the tooth should be the unit of caries enumeration rather than the tooth surface. The reasons for this are as follows: (1) using surfaces, comparisons between different studies are made very difficult since surfaces are not counted in a uniform way for different studies or even in the same mouth (an extracted molar may count for 5 surfaces but does a lost incisor also count for 5 surfaces?); (2) in many studies the subjects receive routine dental care from their private dentists, including filling of proximal cavities (it is not always possible to determine whether 2 surfaces were originally attacked by caries or whether the standard proximo-occlusal cavity preparation extended a one surface lesion to a 2 surface filling); (3) interindividual variation is difficult to assess when dealing with teeth, unless all patients start with the same number and kind of teeth. The variation is even more difficult to assess when surfaces are used. Five attacked surfaces are counted when a tooth is extracted—is this the same as 1 attacked surface of each of 5 teeth?

It is the authors' opinion that x-ray examination should be part of the examination procedure in the dental caries clinical trial. The subjects should have these x-rays (usually bite-wings) taken a short time before the clinical oral examination is performed so that the examiner can read the films while examining the mouth and thus record caries status more accurately.

Reversals.—One problem occurring in the evaluation of clinical caries studies is that of "reversals." By "reversal" is meant the "disappearance" of a carious lesion on subsequent examination after it had been recorded on a previous examination. Thus, on the first examination there may be charted an occlusal carious lesion on the upper right first premolar, and on the second examination the occlusal surface is intact. This occurrence may be due to an error in recording, a difference in the choice of criteria as to what constitutes a carious lesion, or actual healing of the original lesion. We do not believe this last reason is valid except, perhaps, in rare instances. The second reason can, to a large extent, be reduced to a minimum by carefully indoctrinating the examiners in the criteria to be employed in charting caries.[12] Recording errors will be always with us and may be classified as part of error of measurement. These errors should occur to the same degree in both experimental and control groups, so that the over-all outcome is not affected. This type of error, however, should be kept to a minimum, lest the validity of the observations be open to question. An impartial observer could certainly look askance at a study in which "reversals" occurred as often as a quarter of the time. When the tooth is used as the unit of enumeration the chances of recording error should be less than when the various surfaces are employed.

SUMMARIZING AND ANALYZING THE DATA

Comparison of Average Number of Teeth.—The actual tabulations and calculations for the comparisons of the average number of noncarious permanent teeth in the mouth during the study period for the 2 groups, as well as

for the comparisons of those teeth which became carious during the study period, can be simplified quite a bit by the use of correlation tables. Table I presents such a correlation table illustrating the distribution of 161 individuals using a control dentifrice tabulated simultaneously according to the number of caries-free teeth present at the beginning of the study and erupting during the study period (y, the vertical axis) and the number of teeth becoming carious by the end of the study period (x, the horizontal axis).[13] In setting up such a table, for example, a child who has 10 teeth noncarious at the beginning of the study period or erupting during this time, and who by the end of the study has developed 3 carious teeth, would be tabulated in the cell opposite 10 on the vertical scale and opposite 3 on the horizontal scale. In the final tabulation, 2 children were found to have this double characteristic.

TABLE I

DISTRIBUTION OF 161 CONTROL CHILDREN BY NUMBER OF CARIES-FREE TEETH AT BEGINNING AND ERUPTING DURING STUDY AND NUMBER OF TEETH BECOMING CARIOUS DURING STUDY

No. of Caries-Free Teeth at Beginning and Erupting During Study Period (y_i) (Eligible Teeth)	NUMBER OF TEETH BECOMING CARIOUS DURING THE STUDY PERIOD (x_i)									NO. OF INDIVIDUALS (f)	NO. OF ELIGIBLE TEETH f (y_i)	f $(y_i)^2$
	0	1	2	3	4	5	6	7	8			
0										–	–	0
1	1									1	1	1
2	3									3	6	12
3	3									3	9	27
4	1	–	3	1						5	20	80
5	1	1	1	1						4	20	100
6	7	–	–	2						9	54	324
7	3	3	2							8	56	392
8	15	7	3							25	200	1600
9	5	5	–	5						15	135	1215
10	6	2	1	2						11	110	1100
11	3	3	1	1						8	88	968
12	5	1	1						1	8	96	1152
13	4	3	1							8	104	1352
14	2	2								4	56	784
15	1	1	1							3	45	675
16	–	1	2							3	48	768
17	2	2	–	–	1				1	6	102	1734
18	2	–	2	2				1		7	126	2268
19	2	2	2			1				7	133	2527
20	1	1	1	1	2	–	2			8	160	3200
21	–	1	–	2	1					4	84	1764
22	1	1	2	2	–	–	1			7	154	3388
23	2	–	–	–	1					3	69	1587
24				1						1	24	576
No. of individuals (f)	70	36	23	20	5	1	3	1	2	161	1900	27594
No. of carious teeth f(x_i)	0	36	46	60	20	5	18	7	16	208		
f$(x_i)^2$	0	36	92	180	80	25	108	49	128	698		

While the table illustrated is for all noncarious teeth both present at the start of the study and erupting during the study period, similar tables can be constructed for only those teeth present at the start of the study, and for those teeth erupting during the study period. Such separate tables for both the experimental dentifrice group and for the control dentifrice group can be constructed at different stages of the study.

In Table II is presented the distribution of 216 children using the experimental dentifrice, classified according to the same criteria as those in Table I over the same time period. The advantage of such a method of tabulation readily becomes apparent in the ease of summarization of these data. Thus, in Table I, the number of children who have 8 teeth eligible for caries attack is found by adding horizontally opposite 8 on the vertical scale: $15 + 7 + 3 = 25$ individuals. Similarly, with 13 eligible teeth there are $4 + 3 + 1 = 8$ individuals. The total number of individuals is readily obtained as the total of the column denoted as "number of individuals"; thus, $1 + 3 + 5 + \ldots + 1 = 161$ individuals ($= \Sigma f$ or N). To obtain the figures listed in the second column at the right of the table as "number of eligible teeth," it can be seen that there is 1 child with 1 eligible tooth, 3 children with 2 eligible teeth, 3 with 3, etc., so that $1 \times 1 + 3 \times 2 + 3 \times 3 + 5 \times 4 + \ldots\ldots + 1 \times 24 = 1{,}900 = \Sigma f(y)$. Thus, we can see that in the control group, 161 children had 1,900 noncarious teeth eligible for caries attack. Referring to Table II, we find that 216 children had a total of 2,630 noncarious teeth eligible for caries attack. The average number ($\bar{y}$) of eligible teeth is thus 12.18 for the experimental group and 11.80 for the control group.

TABLE II

DISTRIBUTION OF 216 CHILDREN IN EXPERIMENTAL GROUP BY NUMBER OF CARIES-FREE TEETH AT BEGINNING AND ERUPTING DURING STUDY, AND NUMBER OF TEETH BECOMING CARIOUS DURING STUDY

Number of Caries-Free Teeth at Beginning and Erupting During Study Period (y_2) (Eligible Teeth)	NUMBER OF TEETH BECOMING CARIOUS DURING THE STUDY PERIOD (x_2)							NO. OF INDIVIDUALS (f)	NO. OF TEETH $f(y_2)$	$f(y_2)^2$
	0	1	2	3	4	5	6			
0	1							1	0	0
1	–							–	0	0
2	4							4	8	16
3	–	1						1	3	9
4	5	–						5	20	80
5	4	2						6	30	150
6	11	4						15	90	540
7	5	–	2	1				8	56	392
8	18	4	2	–				24	192	1536
9	14	5	3	1				23	207	1863
10	10	3	3	–	1			17	170	1700
11	11	5	–	–		1	1	18	198	2178
12	7	1	–	–	1			9	108	1296
13	3	3	1					7	91	1183
14	9	–	–					3	42	588
15	5	1	–					10	150	2250
16	4	–	–					5	80	1280
17	6	5	–					9	153	2601
18	3	5	1					12	216	3888
19	4	2	2	2	1			10	190	3610
20	3	4	1	2				11	220	4400
21	–	1	2	1				7	147	3087
22	–	2	1	2				5	110	2420
23	–	–	1	1				2	46	1058
24	–	2	1					3	72	1728
31	1	–						1	31	961
No. of individuals (f)	131	50	20	10	3	1	1	216	2630	38814
No. of eligible teeth $f(x_2)$	0	50	40	30	12	5	6	143		
$f(x_2)^2$	0	50	80	90	48	25	36	329		

In order to calculate the standard deviation of the number of eligible teeth, we first need to calculate $\Sigma f(y^2)$, as given in the last column of Table I. Thus, $1 \times (1)^2 + 3 \times (2)^2 + 3 \times (3)^2 + 5 \times (4)^2 + \ldots + 1 \times (24)^2 = 27{,}594$. Similarly, in Table II, we have 38,814. The standard deviation (σ) for each group can be calculated from the formula:

$$\sigma_y = \sqrt{\frac{\Sigma f(y^2)}{N} - \bar{y}^2}$$

$$\text{where, } \bar{y} = \frac{\Sigma fy}{N}.$$

The comparison of means then proceeds as follows[14]:

Control Group (1)

$$\bar{y}_1 = \frac{1900}{161} = 11.80$$

$$\sigma^2_{y_1} = \frac{27594}{161} - (11.80)^2 = 32.151.$$

Experimental Group (2)

$$\bar{y}_2 = \frac{2630}{216} = 12.18$$

$$\sigma^2_{y_2} = \frac{38814}{216} - (12.18)^2 = 31.342.$$

The standard error of the difference in means is calculated as:

$$\sigma_{\bar{y}_1 - \bar{y}_2} = \sqrt{\frac{\sigma^2_{y_1}}{N_1} + \frac{\sigma^2_{y_2}}{N_2}} = \sqrt{\frac{32.151}{161} + \frac{31.342}{216}} = \sqrt{0.3448} = 0.59.$$

The ratio of the difference in means to its standard error (Dev/σ) is:

$$\text{Dev}/\sigma = \frac{\bar{y}_1 - \bar{y}_2}{\sigma_{\bar{y}_1 - \bar{y}_2}} = \frac{11.80 - 12.18}{0.59} = \frac{0.38}{0.59} = 0.64.$$

Since the ratio is less than 2, we conclude that the difference between the average number of teeth eligible for caries attack in the experimental and control groups is statistically not significant. Thus, there is reason for considering these 2 groups comparable at the end of the study period with respect to initial eligibility for caries attack. Such comparisons can be made separately for teeth present at the initial examinations as well as for the teeth erupting during the study period.

The comparison of major interest is that between the 2 groups with respect to the number of decayed teeth developing during the study period. This may be made by doing the same operations on x (the number of teeth becoming carious during the study period) as were illustrated on y (number of eligible teeth). Thus, the total number of new carious teeth in the control group is given in the next to the last row of Table I as: $70 \times 0 + 36 \times 1 + 23 \times 2 + \ldots + 2 \times 8 = 208 = \Sigma f(x)$. Similarly for the experimental group, in Table II the total number of new carious teeth is 143. The average number of new carious teeth in the control group is 1.29 and in the experimental dentifrice group is 0.66. Using the $\Sigma f(x^2)$ for each group (last row in Tables I and II) we calculate $\sigma^2_{\bar{x}_1}$ and $\sigma^2_{\bar{x}_2}$ to be 2.666 and 1.085, respectively. Consequently, $\sigma_{\bar{x}_1 - \bar{x}_2}$ is 0.15, and the ratio of the difference between the two means and its standard error is 4.2. Hence, the difference is statistically significant, i.e., can be exceeded by the action of chance factors alone less than 1 per cent of the time.

Apparently, then, the test dentifrice has effected a reduction in the average number of newly decayed teeth. Since the average of the experimental group was 0.63 teeth less than that of the control group, the former had $\frac{0.63}{1.29} \times 100 =$ 48.8 per cent less new carious teeth.

Comparison of Average Attack Rates.—If the study groups were selected as was described in a previous section of this paper, the distribution of eligible teeth should be similar for the test and control groups—at least within chance limits. In order to iron out any possible difference in the distributions, there may be some advantage in calculating an attack rate for each individual. The comparison can then be made between the two groups in terms of the average attack rate. The comparison in terms of attack rates would also take advantage of the fact that the number of newly decayed teeth in the mouth at the end of the study bears some relation to the number of eligible teeth present. Individual attack rates can readily be calculated from the correlation tables. Thus, for example, in Table I there are 2 individuals who have 10 eligible teeth of which 3 became carious during the study period. For these 2 individuals, the attack rate is then 30 per cent. Proceeding in this manner, for the 161 individuals in Table I we find an average attack rate ($\bar{x}_1$) of 11.0 per cent, with a standard deviation (σ_{x_1}) of 14.6 per cent, and a standard error ($\sigma_{\bar{x}_1}$) of 1.1 per cent. For the 216 individuals in Table II, the average attack rate ($\bar{x}_2$) is 5.4 per cent, with a standard deviation (σ_{x_2}) of 9.2 per cent and a standard error ($\sigma_{\bar{x}_2}$) of 0.6 per cent. The standard error of the difference ($\sigma_{\bar{x}_1 - \bar{x}_2}$) is 1.3 per cent. Consequently the ratio of the difference between the average attack rates ($\bar{x}_1 - \bar{x}_2$) to the standard error of the difference is 4.3 (Dev/σ). In general, it would be expected that the approach in terms of attack rates is somewhat better than in terms of average number of teeth. However, it is questionable whether there is sufficient gain to offset the more tedious calculations necessary with this method of comparison.

Comparison of Proportions Not Attacked by Caries.—A simpler (but less complete) type of analysis would consist in determining what proportion in each group did not become attacked with the disease in the study period, in the treated and control groups. This approach is relevant since the advance of dental caries in the individual is often spasmodic with relatively long periods of inactivity.[15]

From Tables I and II (summarized in the fourfold Table III) we find that 70 children out of the 161 in the control group (43.48 per cent) and 131 out of 216 in the experimental group (60.65 per cent) developed no new carious teeth during the study period. This is a difference of 17.17 per cent or a relative difference between these two percentages of $\frac{17.17\%}{43.48\%} \times 100 = 39.49$ per cent. The difference (17.17 per cent) can be tested for statistical signifi-

cance by either normal curve technique or the equivalent χ^2 analysis. By the normal curve technique,[14]

$$\sigma_{p_1 - p_2} = \sqrt{\frac{(53.32\%)(46.68\%)}{161} + \frac{(53.32\%)(46.68\%)}{216}} = 5.15\%.$$

$$\text{Dev}/\sigma = \frac{p_1 - p_2}{\sigma_{p_1 - p_2}} = \frac{17.17\%}{5.15\%} = 3.33.$$

The difference is statistically significant since the probability of exceeding it is less than 1 per cent. Thus, a significantly greater proportion of children were protected against caries attack by the test dentifrice than by the control dentifrice.

TABLE III

FOURFOLD TABLE OF PROPORTION OF CHILDREN REMAINING CARIES FREE AFTER USING A CONTROL DENTIFRICE OR AN EXPERIMENTAL DENTIFRICE DURING THE STUDY PERIOD

DENTIFRICE USED	NO. CHILDREN REMAINING CARIES FREE	NO. CHILDREN CARIES ATTACKED	TOTAL	% REMAINING CARIES FREE
Control	70	91	161	43.48
Experimental	131	85	216	60.65
Totals	201	176	377	53.32

It must be pointed out that the average comparison of proportions is not independent of that of the average number of decayed teeth. This is due to the fact that a high proportion of zeros will usually result in a low average. A further comparison which might on occasion, be of some interest would be that between the average number of new carious teeth among those individuals attacked. Using the data in Tables I and II, the averages are 2.29 (control) and 1.68 (experimental) and the standard error of the difference is 0.19. The ratio (Dev/σ) is 3.21, so that the difference is statistically significant. Thus, this test dentifrice not only was effective in preventing new caries, but also made the attacks on the average individual so attacked, less severe. This latter test is not independent either of the test on proportions or of the original test on averages using the whole distribution.

A Method of Comparison of Specific Teeth or Surfaces.—Instead of studying the whole tooth population in each individual's mouth, the effect of a caries-inhibitory agent can be studied on a specific tooth. Thus, for example, the number of children with a noncarious lower left first molar which became carious during the study can be compared for the 2 dentifrice groups. The final evaluation would be made in terms of proportion of these teeth in each group which became carious. The analysis would be similar to that illustrated previously for the nonreactors. The advantage of such a comparison would be that the same tooth would serve as the focus of caries inhibition for all the individuals in the study.

A similar type of comparison of possibly greater interest would be that involving a specific surface on a specific tooth. For example, the investigator can select the mesial surface of the upper right first molar as the surface to be studied. Thus, of 191 cases with this particular tooth surface noncarious

at the start, at the end of one year 20 (or 10.47 per cent) had become carious after using dentifrice "A." In a comparable group of children using dentifrice "B," 15 of 174 (or 8.62 per cent) noncarious surfaces had become carious after one year. In this situation, each subject contributes only one occurrence to the total.

The data can be summarized in a fourfold table (Table IV). The difference between the occurrrence of caries on the specific tooth surfaces in question in each group can be tested for statistical significance by either χ^2 or the normal curve.

TABLE IV

OBSERVED NUMBER OF CHILDREN ACCORDING TO CARIES STATUS OF MESIAL SURFACE OF UPPER RIGHT FIRST MOLAR AT END OF 1 YEAR (f)

DENTIFRICE GROUP	CARIOUS	NONCARIOUS	TOTAL	% CARIOUS
"A" dentifrice	20	171	191	10.47
"B" dentifrice	15	159	174	8.62
Totals	35	330	365	9.59
A. Theoretical number of children according to caries status of mesial surface of upper right first molar at end of 1 year, assuming no difference in results (f')				
"A" dentifrice	18.3	172.7	191.0	9.59
"B" dentifrice	16.7	157.3	174.0	9.59
Totals	35.0	330.0	365.0	9.59
B. (f – f')				
"A" dentifrice	+1.7	–1.7	0	
"B" dentifrice	–1.7	+1.7	0	
Totals	0	0		

In a χ^2 analysis,[14] a table of theoretical frequencies (f') is obtained on the assumption that there is no difference between the proportion of new specific carious surfaces in each group. Thus, 9.59 per cent of the 191 surfaces in group "A" and 9.59 per cent of the 174 surfaces in group "B" would become carious (Table IV, *A*). The difference between the observed (f) and theoretical frequencies (f') for each cell appears in Table IV, *B*. The contributions to χ^2 are obtained by dividing the square of each difference by the appropriate theoretical value, as $(f - f')^2/f'$. By adding the contributions we obtain χ^2 as 0.36. χ^2 of 6.64 is equaled or exceeded just 1 per cent of the time by chance factors alone when there is one degree of freedom as in the fourfold table. Since our value of χ^2 is much less than 6.64, the difference between the number of children with an originally noncarious mesial surface of the upper right first molar which became carious in dentifrice A group and in dentifrice B group could readily be ascribed to chance. The difference is statistically not significant.

By studying specific surface caries in several selected sites, information can be obtained on surface caries activity in each study group. In the experiment cited here, similar surface comparisons were made on the mesial surface of the lower left first molar, distal surface of the upper right central incisor, and the mesial surface of the upper left lateral incisor. In no instance was there any statistically significant difference between the results with either dentifrice group.

Cooperative Studies.—A common occurrence in studies of the clinical effectiveness of possible caries-inhibitory agents has been the inability of independent investigators to reproduce the results of other workers. On occasion, one worker finds that an agent is effective. The sponsoring organization then introduces this agent to the public. Further studies by other workers then show no effect with the new agent. By that time, 1, 2, or even more years have passed and the public and profession both have become disillusioned.

A more profitable approach might be to have the sponsoring organization undertake a clinical trial at several different places at the same time. Thus, for example, grants-in-aid could be given to 3 or 4 institutions which would then set up identical studies (or as close to this goal as possible). Over-all coordination might rest with one individual, but the planning and execution should be a cooperative endeavor. In this way repetitions of the experiment could be performed.

Attempts have been made along these lines, but they have not been based upon the ideas set forth above. Where such studies have been attempted, the aim has been to obtain larger samples with whatever gain might result therefrom. Cooperative studies may, of course, become somewhat unwieldy and, in addition, proper criteria for obtaining comparability between test and control groups, and among the various locations, may not be readily adhered to.

The advantage of repeating the experiment in a number of different places is to be able to draw conclusions applicable to a much wider population. It is also reassuring if the results are reproducible.

Such cooperative studies are sometimes analyzed by pooling the various test groups and the various control groups and then testing the significance of the difference between the two averages. Since caries activity often varies in different locales, and since the effect of the test agent, even if consistent in direction, may vary in magnitude from place to place, this type of analysis may not be correct.

The suggested approach to the analysis of such data would be, first, to analyze and interpret the data from each particular place. The next step would be to examine whether the effect of the test agent is consistent in direction and magnitude. The third would be to compare the average for the test group with that for the control group, over the whole series of experiments. The appropriate method for making this comparison depends on whether the effect of the test agent can be considered merely a sample. The latter is usually the case. The arithmetical aspects of the comparisons suggested above would be greatly simplified if the size of all the groups in the different areas were the same, or at least if the test and control groups in a given area were equal in size. Otherwise, some cumbersome weighting procedure would be necessary.

While the following cooperative study[17] does not conform to all the suggested criteria, the data can be used for illustrative purposes. Moreover, the measurements are solely of surfaces obtained by radiographic evidence alone. The data pertain to the effectiveness of a new caries-inhibitory agent incorporated into a dentifrice. Students from 3 universities and employees of a publishing company were used. Table V gives the data on new caries surfaces for the placebo group and for the test group, both groups brushing morning and evening. Unfortunately, the distribution of individuals according to number of carious surfaces is not given for the various test and control groups.

TABLE V
AVERAGE NUMBER OF CARIOUS SURFACES IN PLACEBO GROUP AND TEST GROUP IN FOUR LOCALITIES

	PLACEBO			TEST			DIFFERENCE
LOCATION	NO. OF CASES	AVERAGE AGE	AVERAGE NO. OF CARIOUS SURFACES	NO. OF CASES	AVERAGE AGE	AVERAGE NO. OF CARIOUS SURFACES	DECREASE IN AVERAGE NO. OF CARIOUS SURFACES
Gainesville	105	20	0.61	154	19	0.34	0.27
Miami	136	22	0.85	161	22	0.24	0.61
Meredith Cty.	294	23	0.67	213	26	0.30	0.37
Drake	151	19	0.76	172	19	0.18	0.58
Totals	686	21	0.716	700	22	0.266	0.450

Thus, the effect of the test agent cannot be evaluated separately for each locality. From the pooled data on the placebo (control) groups and on the test groups (Table VI), we find that the ratio of the difference in averages (0.45) to its standard error is 10, so that by this method the difference is statistically significant. This method is not appropriate because it ignores the fact that the test and control groups are not represented to the same degree in the various places, that caries activity may vary from place to place, and that the effect of the test agent may also vary from place to place.

TABLE VI
DISTRIBUTION OF 686 INDIVIDUALS IN A PLACEBO GROUP AND 700 IN A TEST GROUP ACCORDING TO NUMBER OF NEW CARIOUS SURFACES

NUMBER OF NEW CARIOUS SURFACES	NO. OF INDIVIDUALS	
	PLACEBO	TEST
0	374	563
1	190	104
2	85	23
3	20	5
4	15	4
5	1	1
6	1	
Totals	686	700
Average	0.716	0.266
Standard deviation	0.981	0.633

While the data available do not indicate any significant variation in caries activity from region to region, they do indicate that the effect of the treatment is not uniform (shown by analysis of variance[14]). Consequently, the significance of the average difference of 0.45 should be assessed in terms

of the variation of the difference from locality to locality. The 4 differences (Table V) have a standard deviation of 0.165 (using 3 rather than 4 as the divisor). The standard error of the average difference is $0.165/\sqrt{4} = 0.082$, so that the ratio of the difference to its standard error is 5.5. This ratio is referred to the t table with 3 degrees of freedom instead of to the normal curve, giving a probability of approximately 1 per cent. Thus, even on this basis the effect of the test agent is demonstrated. This suggested method of assessing the evidence from a group of individual experiments appears to be satisfactory in general, except that it is not very sensitive if there are only a few experiments. It should be noted that the suggested method gives all of the differences the same weight, whereas logically they might be weighted in some manner according to the variable number of observations in the control and test groups. The procedure of weighting is somewhat tedious and usually does not yield results very different from the unweighted approach.

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DIMENSIONAL CHANGE IN WAX PATTERNS DURING SETTING OF GYPSUM INVESTMENTS

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IT HAS been reported[1] that a large variation exists in the size of inlay castings even when they are made by technics more standardized than those which could be expected in a dental office. It is possible that much of this variation occurs between the time the pattern is placed in the investment and when the gold enters the mold. One of the major factors involved during this time interval is the effect of the setting and hygroscopic expansion of the investment upon the size or shape of the mold.

As early as 1912, Price[2] recognized that there might be some distortion of the pattern due to expansion of investment. Much later, experimental evidence was obtained to indicate that restriction by the ring and by the pattern could seriously affect the setting expansion of gypsum products.[3, 4] Taylor[5] also indicated that distortion would occur.

Smyd,[6] by mathematical calculations, suggested the pattern of distortion which might take place. However, the only detailed study in this area is that of Jorgensen.[7] The technic employed led to some interesting observations. However, the investigation included only one investment used in this country and although variation in mold size was noted, no detailed work was carried out to investigate the degree of distortion, its relation to the investing technic used, or to other variables encountered during the investing procedure. It was thought that an experimental method which gave, in one measurement, the actual dimension of the investment mold may be more accurate.

Therefore, the purpose of this research was to evolve a better method of investigation, to determine the degree of distortion of wax patterns by the setting investment, and the effect of certain other variables on this distortion.

PROCEDURE

The patterns used were formed on a stainless steel MOD die. Although this die conforms to the basic type recommended by the Bureau of Standards, it has thicker occlusal and proximal portions, with the proximals being somewhat shorter than those usually found in dental practice.

The occlusal and gingival surfaces were engraved with sharp lines in order that accurate measurements could be made by means of a Gaertner comparator microscope in a mesiodistal direction, between the areas corresponding to the

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axiopulpal line angles and the gingival floors (Fig. 1). It was found that with this instrument the reading accuracy was 0.05 per cent. Wax was melted and poured into the warmed die. The wax was held under heavy pressure until it reached room temperature. A series of patterns formed in this way were measured after 10 minutes and the measurements were compared with those

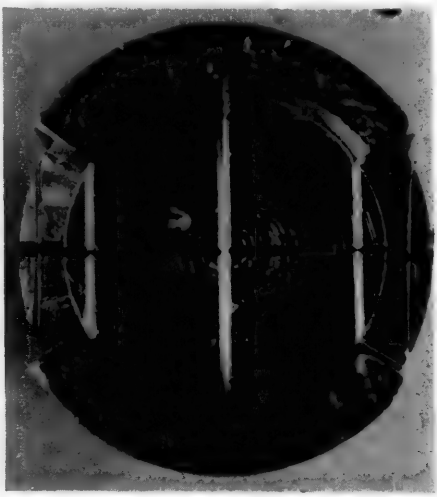

Fig. 1.—The steel die showing the position of the engraved lines.

Fig. 2.—A sectioned investment specimen.

of the steel die. The change in dimension, if any, fell beyond the range of accuracy of the microscope. It was therefore presumed that no distortion had occurred prior to investing. However, change in dimension did occur in a direction corresponding to the buccolingual diameter of the occlusal portion; thus, measurements in this direction were not used. Further verification of this statement will be made in experimental work in the latter part of the paper.

The patterns were vacuum-invested. The invested patterns were allowed to set, with the crucible former as a base, for a period of 24 hours. The crucible former, sprue pin, and inlay ring were removed. Investment was carefully cut away until the occlusal surface of the pattern was exposed. The mold was also sectioned midway between the occlusal and gingival floors (Fig. 2). The wax was removed by solution in warm carbon tetrachloride. It was found that removal of the wax with hot water dissolved so much of the investment surface that the sharp detail of the lines was lost. The use of carbon tetrachloride did not affect the surface or cause any dimensional change in the investment. The distance between the transferred lines on the investment was again measured to 0.001 mm. The difference between the measurements on the die and on the investment thus represented the change in dimension which had occurred in the wax pattern.

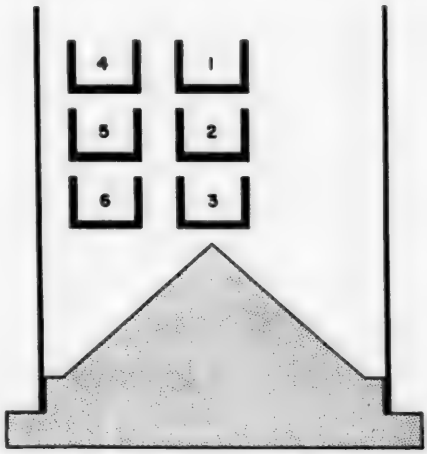

Fig. 3.—Diagram of the areas in which patterns were invested.

Four investing technics were used: (A) a cristobalite investment with a wet asbestos liner; (B) the controlled water addition hygroscopic technic; (C) the warm water bath hygroscopic technic; and (D) an experimental investment* with a setting expansion of 0.06 per cent used in a flexible rubber inlay ring.

Patterns were invested in 6 positions in the ring as diagrammed in Fig. 3. For every position, with a given technic, a minimum of 5 patterns were invested and measured except in the case of the experimental investment where, due to the quantity available, only 3 specimens were made. The standard deviation for the results, shown in Table I, was less than 0.06 per cent.

RESULTS

Distortion.—Distortion is evident when there is a difference in the percentage dimensional change between the occlusal and gingival dimensions of the

*Supplied by Ransom and Randolph Company, Toledo, Ohio.

TABLE I
THE EXPANSION OF THE INVESTMENT IN THE VARIOUS AREAS OF THE RING

	TECHNIC A		TECHNIC B		TECHNIC C		TECHNIC D	
POSITION	OCCLUSAL %	GINGIVAL %	OCCLUSAL %	GINGIVAL %	OCCLUSAL %	GINGIVAL %	OCCLUSAL %	GINGIVAL %
1	−0.10	+0.40	+0.05	+0.07	+0.30	+0.70	−0.25	−0.20
2	+0.08	+0.74	+0.10	+0.84	+0.75	+0.87	−0.23	−0.19
3	−0.01	+0.78	+0.21	+1.00	+0.56	+1.20	−0.22	−0.17
4	−0.48	+0.50	+0.25	+0.44	+0.22	+0.95	−0.50	−0.26
5	−0.23	+0.85	−0.04	+0.80	+0.62	+1.0	−0.13	+0.09
6	−0.24	+0.86	+0.34	+1.05	+0.37	+1.1	−0.08	+0.26

mold. It can be seen from Table I that distortion occurs with all the investments studied. The form which the distortion takes is generally a bending of the occlusal portion of the pattern. There appears to be little change in the angle formed at the junction of the occlusal and proximal parts of the pattern (Fig. 4). In cases where the distortion was severe, a convexity formed on the occlusal surface of the investment and was even visible to the naked eye. Some specimens contracted across the occlusal. This contraction is possibly due to the force of the investment acting on the gingival portion of the

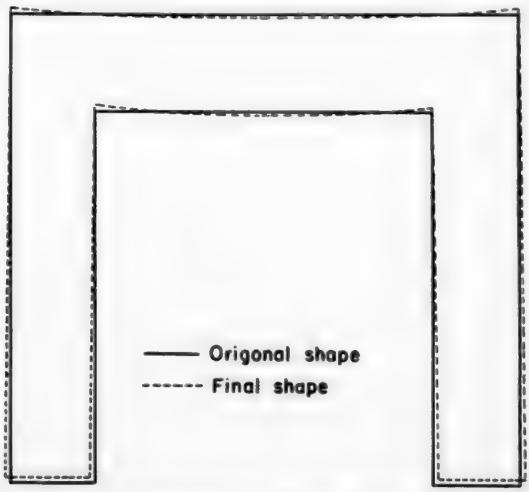

Fig. 4.—Diagrammatic representation of the typical pattern of distortion.

pattern and thus working through the proximal as a lever arm causing compression of the investment adjacent to the occlusal. In no case was there any evidence of fracture of the wax pattern that could be ascribed to the setting expansion.

There is little difference in the degree of distortion between technics A and B. In technic B the distortion is coupled with an increased over-all expansion of the mold. This is to be expected as this technic involves hygroscopic as well as setting expansion.

In technic C the use of a warm water bath at 98° F. has reduced the distortion. This is probably due to a decrease in the physical resistance of the

wax to the expanding investment. However, it is apparent that a temperature in excess of 98° F. would be required to soften a medium grade inlay wax sufficiently to minimize distortion further. It must be recognized that the patterns used were relatively stress free and the use of a warm water bath would produce minimum distortion due to release of this stress. In clinical practice, however, where the internal stress is usually greater, more distortion would probably be evidenced.

When an investment of very low setting expansion is used, a shrinkage of the wax pattern generally occurs (technic D). The degree of distortion is less than in any of the other technics.

It is apparent from these data that although an investment mass may have an over-all expansion, internally the investment may have areas of contraction.

Position of the Pattern in the Ring.—The most obvious fact evident from the results is the variation in expansion and distortion which occurs in different areas of the ring. In general, both expansion and distortion increase as the pattern is placed close to the closed or crucible former end of the ring and also as the pattern is moved toward the side of the ring. The maximum expansion is gained by placing the pattern near the ring and at the closed end.

In technics B and C, where hygroscopic expansion is utilized, there is a marked decrease in expansion at the open end of the ring. This may be due to a weakening of the investment by dilution with water.

To determine whether this effect was due to (1) the orientation of the ring while the investment was setting, or (2) the restriction of the crucible former, technic A was used. The inlay ring was inverted on a glass slide immediately after investing. The results are shown in Table II. It can be seen in the results for the occlusal dimension of the pattern that an increase in expansion occurs as the pattern is moved toward the end of the ring resting downward. This is not entirely true in the case of the gingival dimension and this will be explained in the following part of the paper.

TABLE II
THE EXPANSION OF THE PATTERN WITH THE RING INVERTED DURING SETTING

POSITION	TECHNIC A	
	OCCLUSAL %	GINGIVAL %
1	+0.10	+0.40
2	0.00	+0.86
3	−0.10	+0.85

Lateral Restriction.—The restrictive effect of the metal ring has been the subject of much discussion. To determine its effect 2 series of 10 patterns each were invested in an inlay ring without a liner. In the first series, the patterns were sprued on the occlusal surface and in the second on the proximal surface. The investment used had a setting expansion of 0.3 per cent and its use in an unlined ring is recommended by the manufacturer for patterns for Class I type restorations.

The results are given in Table III. There is very little change in dimension in the occlusal portion. However, a contraction occurs in the gingival area.

TABLE III
EXPANSION OF THE PATTERN WITH LATERAL RESTRICTION OF THE INVESTMENT

POSITION	OCCLUSAL %	GINGIVAL %
Sprued proximally	–0.10	–0.50
Sprued occlusally	–0.15	–0.60

The contraction is somewhat less when the pattern in invested with the gingival dimension at right angles to the open end of the ring (henceforth referred to as the axial direction). The decrease in contraction, however, is not of the same magnitude as the theoretical setting expansion of the investment, not taking into account any increase that might be expected in the axial direction due to the lateral restriction.

When the over-all expansion of the investment in an axial direction was measured, using a dial gauge, it was found to be 0.4 per cent. To determine how this apparent anomaly occurred, small pieces of wax, inscribed with lines, were invested vertically in the top, middle, and crucible former thirds of the ring. Measurements were made and it was found that there was an area of contraction in the center of the ring but expansion occurred near the ends of the ring (Table IV). The expansion at the ends was sufficient to account for the over-all expansion even though contraction had taken place in the center.

TABLE IV
EXPANSION OF THE INVESTMENT IN A LONGITUDINAL DIRECTION

SECTION	DIMENSIONAL CHANGE
Open end	+0.3%
Middle	–0.3%
Closed end	+0.2%

Investment Expansion.—In view of the differences in expansion in various areas of the ring, the physical effect of the many shapes and cross sectional areas of patterns normally encountered and changes in the physical properties of wax due to temperature changes, an experimental method was developed to investigate the behavior of the investment itself.

Annealed 40-gauge B & S nickel wires were placed as nearly as possible parallel along the long axis of an inlay ring. The wires were held parallel and in the same radial plane by a Plexiglas frame. One wire was placed in the center of the ring and 4 others were spaced equidistant so that the last wire was 2 mm. from the asbestos liner in the ring. Measurements were made in a manner such that the distance between the wires in a given plane could be calculated.

A piece of baseplate wax was placed over the bottom of the ring and holes were left to permit free lateral movement of the wires. These holes were filled with petroleum jelly. The inlay ring was held in a vertical position and filled with investment. At the time of the approximate initial set of the investment, the Plexiglas frame was compressed to relieve all tension on the wires.

After 24 hours, the investment specimen was removed and sections were made at right angles to its length. Measurements were taken between the wires

and these were converted into graphic form showing the per cent changes in dimension in the different areas of investment. The presumption was made that the centrally located wire had undergone no movement.

A graph for technic A is shown in Fig. 5. It is interesting to note that there is a correlation between these data and that obtained previously. There is an increase in expansion from the top down to the center of the ring and then a gradual decrease to the closed end. However, the expansion at the closed end is still much greater than at the open end of the ring. The expansion is also greatest in the outer segment of the investment.

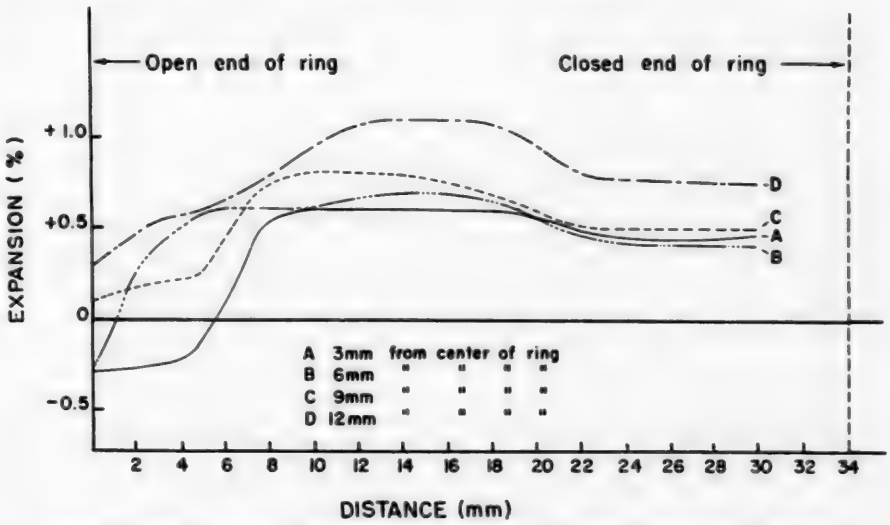

Fig. 5.—Graph showing the lateral expansion of a typical investment specimen.

When this evidence is correlated with the previous data it is evident that when the lateral expansion is greatest, expansion in the other direction is minimized and may even be an actual contraction. At no place is there a very large area of uniform expansion and, considering the size of most inlay patterns, it would be difficult to place the pattern in the ring so that distortion did not occur. It is also apparent that even if the pattern were fabricated from a material offering no resistance to expansion, distortion would still occur. The results for the gingival dimension of patterns, when they are invested and inverted into the ring, is explained by the graph. The relationship of the occlusal and gingival dimensions has been reversed in relation to the increasing expansion toward the bottom end of the ring.

Time at Which Distortion Takes Place.—The time at which the expansion or distortion of the wax pattern began was investigated. Patterns were sprued and placed in the inlay ring. Stainless steel pins, 0.016 in. diameter, with small plates attached at one end and sharpened at the other were passed through small holes in the side of the inlay ring. The plates were carefully attached to the proximal surfaces of the pattern in the gingival and occlusal areas. The

pins were coated with a thin film of petroleum jelly. The pointed ends of the pins were then aligned with the micrometer microscope and measurements taken. The investment was placed around the pattern. The coating of petroleum jelly formed a tube in which the pins could move with relative freedom. The fiducial reading was taken at the start of the mix of investment. When all movement had ceased the pins were again measured.

The movement began within a minute after the initial set had taken place and ceased, with all the investments tested, within an hour after mixing. The change in dimension between the points of the pins corresponded with the change in dimension noted in the first technics described in this paper. This served as a check on the method of wax pattern production and the distortion which may occur during investing.

DISCUSSION

The effect of various basic variables on the distortion of wax patterns has been shown. There are many other factors which would have some effect in changing the degree of distortion and these require further study. Among these are water-powder ratio, spatulation time, size and number of sprues, and ring size. No attempt should be made to correlate the data presented with the final shape or size of the inlay casting.

SUMMARY

Technics have been described for studying wax pattern distortion during the setting of investments. All of the technics investigated produced distortion with the least occurring by use of an experimental investment having a setting expansion of 0.06 per cent. The degree of distortion is dependent upon the position of the pattern in the ring. The distortion begins within 1 minute after the initial set of the investment and reaches its maximum within 1 hour. The investment has unequal dimensional change throughout its mass during setting and this is an ever-present source of distortion.

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MERCURY ANALYSIS BY FLUORESCENT X-RAY SPECTROSCOPY

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SINCE existing methods for mercury analysis of dental amalgam are both laborious and time consuming and cause destruction of the samples used, it was decided to study the feasibility of adapting the principle of fluorescent x-ray spectroscopy for such analysis.

The dependence of mechanical properties (ultimate compressive strength, dimensional changes upon setting, and flow) upon mercury content has been the subject of numerous papers.[2-21] Reports by Gray,[2] Ward and Scott,[3, 4] Ward, Peyton, and Scott,[5] Souder and Sweeney,[6] Gayler,[7] Worner and Anderson,[8] Phillips,[9] Phillips and Boyd,[10] Strader,[11] Stenbeck,[12] and Ware and Docking,[13, 14] base their evaluation of the importance of residual mercury content for the mechanical properties of amalgam upon quantitative chemical analysis. In most instances the methods employed were designed to analyze for silver, copper, tin, and zinc by solution, evaporation, precipitation, and trituration and the mercury content was obtained by computation. Several investigators did not describe the method of analysis. In most instances, rather large quantities (2 Gm. or more) are required to establish the mercury content of a sample with the desired degree of accuracy. Caul and Schoonover[15] described a procedure applicable for use in analysis of smaller samples (0.5 to 0.2 Gm.) for determining the mercury, silver, tin, copper, zinc, and gold in dental amalgams. Although very time consuming, this method has been utilized by Phillips and Swartz[16] and by Ryge, Dickson, Smith, and Schoonover.[17]

A method for mercury analysis based upon evaporation of mercury in a closed system was developed by Crawford and Larson.[18, 19] This method is reported to permit analysis of 10 mg. specimens with an accuracy of 0.02 per cent. Results obtained by this method have been reported by Swartz and Phillips.[20, 21] This high degree of accuracy is not always necessary for practical application, since the procedures involved in mixing and packing amalgam for dental purposes include a very large number of variables, so that amalgam fillings or specimens made under apparently identical conditions may vary as much as 1 to 2 per cent in mercury content, and the mercury content of an amalgam filling may vary as much as 2 per cent in various parts of a single filling.[20] It

The data in this paper are, in part, from a thesis submitted to the Faculty of the Graduate School, Marquette University, in partial fulfilment of the requirements for the degree of Master of Science (Physics Major).

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is therefore felt that any method which allows rapid analysis of specimens with 1 per cent accuracy will suffice for most studies of amalgam composition. Since fluorescent x-ray spectroscopy would appear to be applicable for this purpose, an attempt was made to develop a method for mercury analysis of dental amalgam utilizing the principles of this type of analysis.

METHODS AND MATERIALS

a. *Development and General Theory of the Methods.*—Qualitative and quantitative chemical analysis by fluorescent x-ray spectroscopy was carried out by von Hevesey and Caster in 1923.[22] However, not until high intensity sealed x-ray tubes, efficient Geiger counters, and modern spectrometers became available during the last decade was the method developed for practical application.

Excitation of a material to emit x-rays can be accomplished either by electron (or ion) bombardment, as in an x-ray tube, or by x-ray bombardment. The radiation generated by x-ray bombardment of a material is secondary or fluorescent radiation. Under favorable conditions of excitation, the x-ray emission spectrum of any material consists of a continuous, or "white," radiation of low intensity and a much stronger radiation consisting of wave lengths which are characteristic of the elements present. The characteristic radiation for each element consists of well-defined groups of lines known as the K, L, M, series.

Energies greater than certain minimum voltages, the critical excitation voltage, are required to produce characteristic radiation. According to Moseley's law, corresponding characteristic lines for different elements occur at different wave lengths with a uniform variation throughout the periodic system, depending on the atomic number.

Qualitative analysis of materials can consequently be based on the position of the characteristic lines, and the spectrum of any element is independent of the state of existence of the element (solid, liquid, vapor) and independent of its presence in pure form or in a mixture or chemical compound.

Within a limited range of concentration the intensity of characteristic lines for elements in a given material is directly proportional to the amount of elements present. Consequently, the fluorescent x-ray spectrum emitted from a material can be used to evaluate the relative amount of the components, provided a working curve has been developed on the basis of samples of known compositions.

Only the K radiation at 2 wave lengths, K alpha and K beta, and the L radiation at 3 wave lengths, L alpha-one, L beta-one, and L beta-two are emitted in such intensity that they are of practical interest for qualitative and quantitative analysis.

b. *Equipment.*—The first attempts to develop an instrument for routine x-ray spectrochemical analysis were done in 1946 by the Naval Research Laboratory and, during the following 3 or 4 years, x-ray spectrographs became available commercially. Their basic components are an x-ray tube, a crystal analyzer, a detector system, and a recording device.

Radiation from an x-ray tube of high power rating, usually a tungsten tube, is directed at the specimen which is excited to fluorescence. These secondary rays leave the specimens in all directions. Part of the fluorescent radiation is collimated through a baffle-type collimator to the crystal analyzer. The space lattice of the crystal serves as a 3 dimensional grating, and the x-rays are diffracted according to the Bragg equations: $n\lambda = 2d \sin \theta$, where n = integer, λ = wave length, d = interplanar spacing, θ = angle of incidence. Only x-radiation of wave lengths which satisfy the Bragg equation for a given angle of incidence will be transmitted by the crystal analyzer. Different angles of incidence are obtained by rotating the analyzer, and by gearing the detector device to rotate at twice the angular speed of the crystal, the detector will always be in position to receive the diffracted beam. Using a thin mica crystal as analyzer, the diffracted beam may be focused on the detector slit by bending the crystal cylindrically, and by varying the radius of the focusing circle for different angles of "reflection" by means of a "crystal bending mechanism."

c. *Materials and Sample Preparation.*—Samples for analysis were prepared from triple distilled mercury and a commercial alloy of small particle size. The mercury and alloy used was proportioned by weighing on an analytical balance. All samples were made in triplicate. Table I shows the amounts of mercury and alloy used for each series of samples.

TABLE I

ALLOY (MG.)	MG. Hg	MERCURY (PER CENT)
1,100	900	45
1,000	1,000	50
900	1,100	55
800	1,200	60
700	1,300	65

Mercury and alloy was mixed by thorough trituration in a mechanical amalgamator and was then placed in specimen holders made from autopolymerizing methyl methacrylate in the form of square slabs with a shallow chamber of dimensions 20 by 20 by 1 mm. (Fig. 1).

Preliminary recordings showed that the surface condition of the sample influences the recordings greatly; the samples were consequently stored for a week after which time surface preparation was carried out.

d. *Experimental Procedure.*—The samples were hand ground on No. 400 "Aloxite" paper, placed on an optically flat paper holder, care being taken to avoid heating of the specimen during the grinding operation.

Each sample was placed in the sample holder with the direction of the scratches from the No. 400 paper oriented lengthwise in relation to the incident beam of x-rays. An AEG-50 tungsten tube operated at 50 kv. 50 Ma. was utilized as the primary x-ray source. A mica crystal of 0.03 mm. thickness was used as the crystal analyzer.

After positioning of the mica crystal for optimal focusing conditions, the exact position of the peaks of the mercury L alpha-one, L beta-one, and L beta-two lines were established by repeated scaling at 0.01 degree two theta intervals over the approximate location of the peaks, as determined by slow speed scanning using the spectrogoniometer with direct recording. The minimal scaling times (maximal intensities) were found at 48.78, 40.67, and 40.50 degrees two theta for the L alpha-one, L beta-one, and L beta-two lines, respectively.

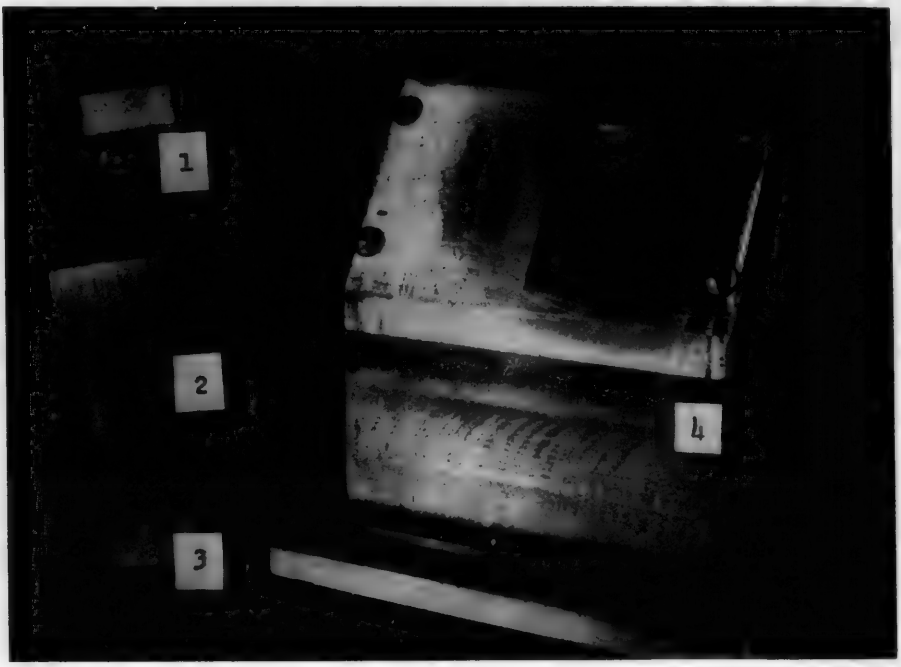

Fig. 1.—Sample Holders and Samples. (1) The window-type sample holder developed for the solid slab-type specimens. (2) A polished amalgam sample in a sample holder. (3) A powdered sample mounted on glass by double-coated Scotch tape. (4) The drawer-type sample carrier with a sample in position for irradiation.

These lines were scaled for all samples. The term "scaling time" connotes the time in seconds which is required to accumulate 2^{14} (16,384) quanta of energy, as recorded by the multichamber Geiger-Mueller counter and its logarithmic amplification system. Counting 2^{14} impulses, the probability factor for correct recording is 0.5 per cent.

Next, each sample was rotated 90 degrees in the sample holder so as to orient the scratches crosswise in relation to the incident beam, and the scaling operation was repeated.

This procedure was again carried out after polishing of the samples on Nos. 1/0 and 3/0 grade emery paper both for lengthwise and crosswise orientation.

Finally the samples were filed into a fine powder, of which 25 or 50 mg. were distributed evenly on double-coated Scotch tape backed by small glass plates (Fig. 1). The scaling operation was repeated for both 25 and 50 mg. powdered samples.

The scaling times thus obtained for the 3 mercury lines were used to compute the number of counts per second.

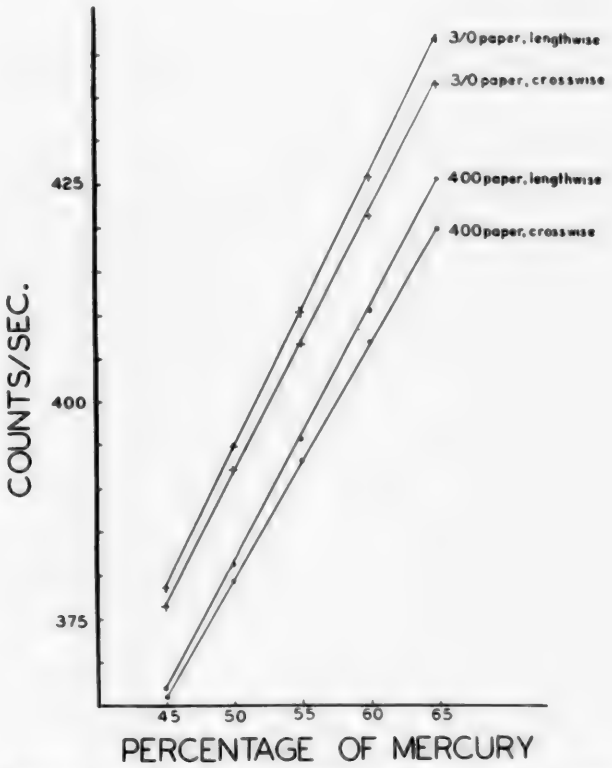

Fig. 2.—Working Curves for Solid Samples. The number of counts per second of energy emitted from the samples is plotted against the known percentage composition of the samples.

RESULTS

Table II gives the scaling times, average scaling times, and corresponding counts per second values for amalgam specimens of 45, 50, 55, 60, and 65 per cent mercury, for lengthwise and crosswise orientation of the scratches from No. 400 "Aloxite" paper and No. 3/0 emery paper. The data given in Table II represent the values obtained for the mercury L alpha-one line at 48.78 degrees two theta. These data are illustrated graphically in Fig. 2.

Table III lists the data obtained on powdered amalgam specimens of known composition. These data are illustrated graphically in Fig. 3.

The reproducibility of the method was controlled by the use of a set of coded samples. Samples of 46, 47, 49, 51, 52, 53, 54, 58, and 61 per cent mercury content were made by a laboratory assistant. The code of the sample designations was kept by the assistant until the x-ray spectroscopic analysis had been carried out. All samples were correctly identified, repeating the experimental conditions used for the establishment of the working curves of Figs. 2 and 3.

The data in Table II indicate that the difference between No. 400 paper and No. 3/0 paper result in a noticeable difference in energy emitted as secondary radiation per unit area, which is consistent with the fact that the finer

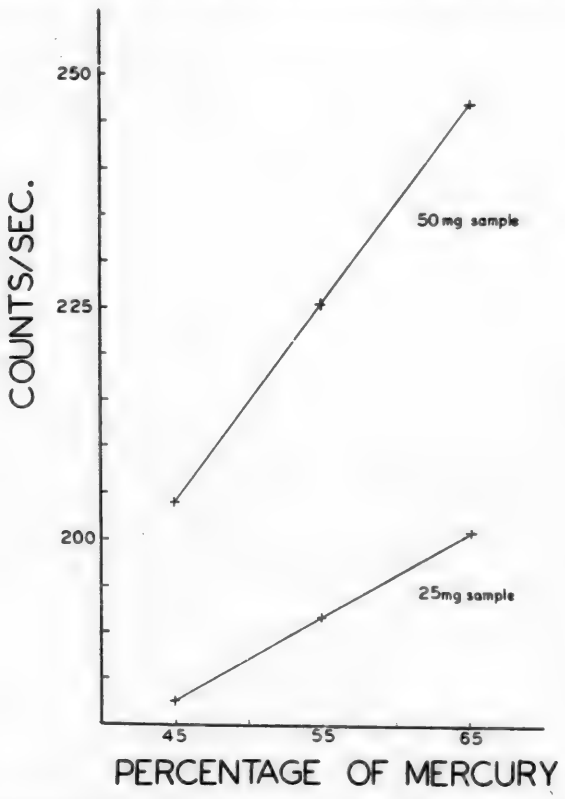

Fig. 3.—Working Curves for Powdered Samples. The number of counts per second of energy emitted from the samples is plotted against the known percentage composition of the samples.

paper leaves a larger part of the unit area in the proper position for the secondary radiation to reach the analyzing crystal. The finer scratches of the No. 3/0 emery paper give rise to consistently higher counting rate than do the No. 400 paper scratches. Also, a slight increase in counting rate is found as a result of lengthwise orientation, as compared to crosswise orientation.

TABLE II

THE SCALING TIMES AND COUNTS PER SECOND FOR THE MERCURY L ALPHA-ONE LINE AT 48.78 DEGREES TWO THETA, FOR LENGTHWISE AND CROSSWISE ORIENTATION OF SAMPLES OF 45 TO 65 PER CENT MERCURY CONTENT, GROUND ON NO. 400 "ALOXITE" PAPER AND NO. 3/0 EMERY PAPER

Hg $L\alpha_1$ $2\theta = 48.78°$	NO. 400 ALOXITE PAPER						NO. 3/0 EMERY PAPER					
	CROSSWISE			LENGTHWISE			CROSSWISE			LENGTHWISE		
% Hg	SC.	A.SC.	CPS	SC.	A.SC.	CPS	SC.	A.SC.	CPS	SC.	A.SC.	CPS
	44.45			44.56			43.40			43.34		
45	44.72	44.76	366	44.38	44.64	367	43.36	43.50	377	43.26	43.26	379
	45.08			44.98			43.74			43.18		
	43.04			42.88			41.65			41.52		
50	42.98	43.15	380	42.99	42.93	382	41.76	41.79	392	41.52	41.50	395
	43.43			42.92			41.96			41.46		
	41.69			41.28			40.25			39.82		
55	41.60	41.67	393	41.42	41.38	396	40.20	40.27	407	39.86	39.90	411
	41.72			41.44			40.36			40.02		
	40.31			39.82			39.00			38.64		
60	40.35	40.25	407	39.84	49.88	411	38.80	38.88	421	38.40	38.46	426
	40.09			39.98			38.84			38.34		
	39.97			38.60			37.42			36.98		
65	39.90	39.90	420	38.42	38.46	426	37.48	37.50	437	37.04	37.07	442
	39.83			38.36			37.60			37.19		

Sc. = Scaling time (seconds) for 2^{14} counts.
A.Sc. = Average scaling time (seconds) for 2^{14} counts.
CPS = Number of counts per second.

The data given on powdered samples (Table III) do not indicate any major difference between the 25 mg. and 50 mg. samples as far as the reproducibility of scaling times are concerned.

It should be pointed out, however, that sample preparation is very critical, and that consistent results between different samples were easier to obtain with 25 mg. samples, most likely because these smaller samples could be spread more uniformly over the tape than the larger quantities.

TABLE III

THE SCALING TIMES AND COUNTS PER SECOND FOR THE MERCURY L ALPHA-ONE LINE AT 48.78 DEGREES TWO THETA FOR POWDERED AMALGAM SAMPLES OF 45, 55, AND 65 PER CENT MERCURY CONTENT, FOR SAMPLES OF 25 AND 50 MG. DISTRIBUTED EVENLY ON SCOTCH TAPE AS SHOWN IN FIG. 1

Hg $L\alpha_1$ $2\theta = 48.78°$	25 MG. POWDER			50 MG. POWDER		
% Hg	SC.	A.SC.	CPS	SC.	A.SC.	CPS
	89.40			79.95		
45	90.20	89.67	183	80.55	80.31	204
	89.40			80.42		
	85.48			73.96		
55	85.38	85.46	192	73.34	73.76	225
	85.52			73.98		
	81.51					
65	81.70	81.49	201	66.24	66.42	247
	81.26			66.44		

Sc. = Scaling time (seconds) for 2^{14} counts.
A.Sc. = Average scaling time (seconds) for 2^{14} counts.
CPS = Number of counts per second.

As a result of the sensitivity of the method, it is necessary that samples to be analyzed be treated in the same manner as those from which the working curve was established. The chief advantage of the method is that once a working curve is established, the analysis of an unknown sample can be carried out in a matter of a few minutes. If proper specimen preparation is observed, the mercury content may be established to the nearest 1 per cent. This accuracy compares favorably with the method described by Caul and Schoonover,[15] but is not comparable to the accuracy obtainable by the method of Crawford and Larson[18] reported to give the mercury content with an accuracy of 0.02 per cent. Since analysis to the nearest 1 or 0.5 per cent will suffice for most routine determinations of residual mercury, the method here described is therefore considered valid for such purposes.

Preliminary recordings revealed that the operational conditions of the spectrometer, counter, and amplification system are also extremely critical. In order to obtain reproducible results, a warm-up period for the electronic equipment of at least one-half hour was found necessary. Also, variations were observed from day to day in the absolute counting rates of any one sample; however, the relative values were maintained. This indicates that in order to utilize the method for routine analysis, one must constantly re-establish the exact position of the working curves on the basis of a few samples of known composition.

It should be noted that recent developments in the field of x-ray spectroscopy include the use of krypton-filled counter tubes or proportional counters with Mylar side window and continual flow of 90 per cent argon and 10 per cent methane gas as well as digital printers and electronically controlled timing units. The use of such equipment permits recording of scaling times for any desired number of counts up to 10^8 counts, thus reducing the statistical and experimental errors existing when the number of counts is limited to 2^{14}.

CONCLUSIONS

1. A method for mercury analysis of dental amalgam based upon the principle of fluorescent x-ray spectroscopy has been developed and working curves have been established for such analysis.

2. The method provides for rapid mercury analysis to the nearest 1 per cent within the range from 45 per cent to 65 per cent mercury content.

3. Specimen preparation is very critical, so that accuracy in mercury analysis requires an exacting technic of specimen preparation as well as extreme care in controlling the many variables inherent in the electronic equipment.

4. The working curves must repeatedly be checked by the use of standard samples of known composition.

5. The statistical and experimental errors can be reduced by increasing the scaling time.

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SOLDER JOINTS ON PLATED RUSTLESS ALLOYS

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WHEN metals or alloys cannot ordinarily be soldered, it has long been accepted practice to make an electrochemical deposition of such materials as copper, nickel, silver, or gold on the solder-resistant surface.

Bonds between various types of rustless alloys used in the mouth have been notoriously unreliable. In previous studies, the authors have investigated various aspects of this problem. In the continuing study by the authors into the nature of the bond between rustless alloys and solder, many facets remain to be explored. A classification of the types of bond which can take place between metals or alloys is in order.

Class I:	Autogenous soldering
Class II:	Soldering by solution
Class III:	Soldering by wetting
	a. Adsorption
	b. Diffusion
	c. Leaching
Class IV:	Electrochemical deposition

In this study, an examination was made of solder joints where solder was melted over a thin electrochemical deposition of gold on rustless alloy. By this procedure, a combination of Class II, III, and IV bonds between them was accomplished simultaneously.

The selection of the 3 types of rustless alloys studied in this series was made both on the basis of the widespread clinical use of 18-8 austenitic steel type 304 and 80 nickel-20 chromium in orthodontic practice and of the superior physical properties of 17-7PH austenitic steel which may be hardened by heat treatment (Tables I and II).

TABLE I

17-7PH STEEL—CHEMICAL COMPOSITION*

Carbon	0.09% maximum
Manganese	1.00% maximum
Phosphorus	0.04% maximum
Sulfur	0.03% maximum
Silicon	1.00% maximum
Chromium	16.00-18.00%
Nickel	6.50-7.75%
Aluminum	0.75-1.50%

*Armco Steel Corporation, Middleton, Ohio, Nov. 1, 1954, Product Data Bulletin, p. 2.

Presented at a meeting of the Dental Materials Group of the International Association for Dental Research, Atlantic City, N. J., March, 1957.

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TABLE II

AVERAGE MECHANICAL PROPERTIES*

	HARD			SOFT		
	YIELD STRENGTH 1,000 PSI	TENSILE STRENGTH 1,000 PSI	ELONGATION (PER CENT)	YIELD STRENGTH 1,000 PSI	TENSILE STRENGTH 1,000 PSI	ELONGATION (PER CENT)
18-8 austenitic type 304	160	185	8	30	85	50
17-7PH austenitic	340	345	7	35	115	40
	hardened at 900°F.±10°F. for 1 hour and air quenched					

*The International Nickel Company, Inc., New York 5, N. Y., 1951, Properties of Some Metals and Alloys, pp. 15, 16.

METHOD

The methods of preparation of the plated materials follow*:

a. 18-8 austenitic steel wire was anodically cleaned in a solution made up of 10 per cent sulfuric acid and 1 per cent hydrofluoric acid at 5 volts for 10 seconds. The wire was gold plated in the gold cyanide solution at 160°F. for 5 minutes at 6 volts.

b. 17-7PH austenitic steel band material was rolled in order to take out a curvature on one side due to sandblasting with high air pressure. After rolling, which reduced the thickness approximately .0005 to .00075 in., the strips were washed in soap and water and lightly sandblasted with fine sand. Gold plating was done in a constant temperature at 180°F. for 20 minutes at 7½ volts. The gold cyanide solution was made up of 1.218 Gm. gold cyanide and 20 Gm. potassium cyanide per liter.

c. 17-7PH austenitic steel wire was anodically cleaned in the 10 per cent sulfuric acid and 1 per cent hydrofluoric acid solution at 5 volts for 10 seconds. The wire was nickel flash-plated in Watts solution for 5 seconds at 6 volts followed by gold plate in the gold cyanide solution at 160°F. for 5 minutes at 5 volts.

d. 80 nickel-20 chromium wire was anodically cleaned in the 10 per cent sulfuric acid and 1 per cent hydrofluoric acid solution at 5 volts for 5 seconds. The wire was nickel flash-plated for 5 seconds at 6 volts in Watts solution followed by gold plate in the gold cyanide solution at 160°F. for 5 minutes at 5 volts.

Crevice corrosion was demonstrated after both chemical and electrical passivation.† Since it was concluded that passivation of rustless alloys might best precede soldering, the unplated samples were passivated for 20 minutes in the following solution prior to soldering: 8 Gm. ferric sulfate (anhyd) 4 Gm. sodium fluoride, 10 c.c. sulfuric acid (conc.), and 90 c.c. water.

The solder joints were prepared using Lynco solder which is made up of silver 40 per cent, copper 30 per cent, zinc 28 per cent, and nickel 2 per cent.

*Acknowledgment is made to A. Bosna of Jelenko & Co., for gold plating rustless alloys supplied by him to the authors.

†Reported at Dental Materials Group, International Association for Dental Research, March 25, 1956.

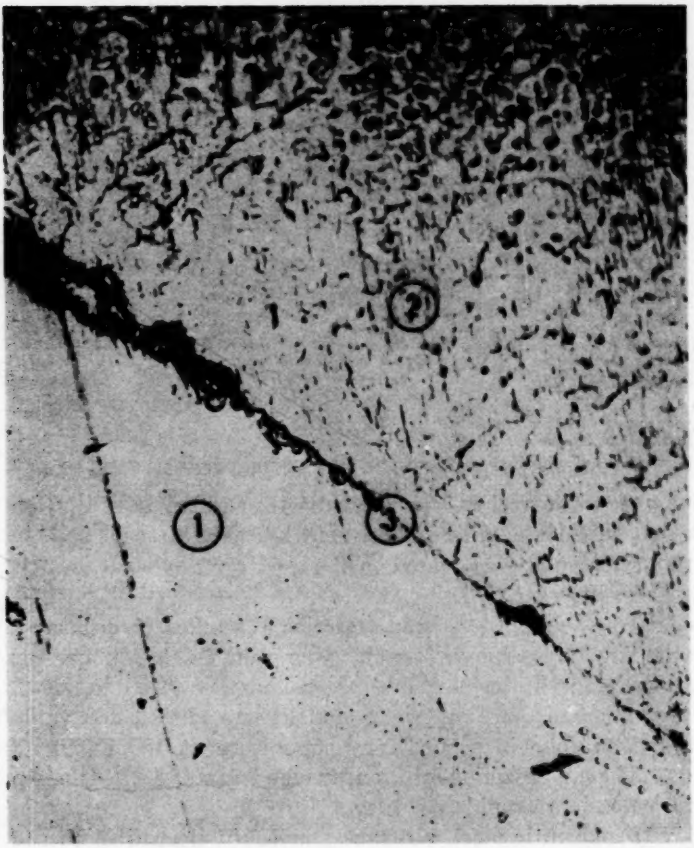

Fig. 1.—17-7PH austenitic band material ①, gold plated for 20 minutes, soldered with 450 fine gold solder ②, then passivated for 20 minutes prior to mounting and polishing, followed by a potassium cyanide etch of the mounted and polished joint. Crevice corrosion appears readily to be seen along with islands of plate ③ which appear not to be dissolved in the solder. (Orig. mag. ×870.)

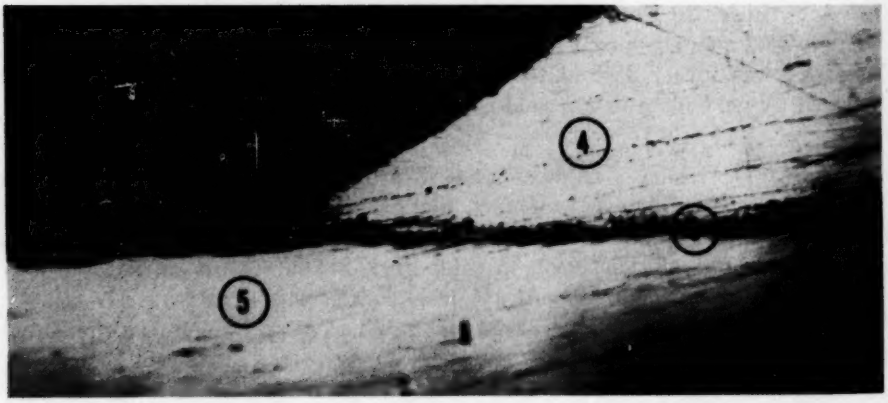

Fig. 2.—Joint of silver solder ④ on 17-7PH austenitic steel wire ⑤, flash-plated for 5 seconds with nickel, followed by gold plate for 5 minutes, mounted, polished, followed by potassium cyanide etch. The gold plate appears to be incompletely dissolved in the solder ⑥. (Orig. mag. ×870.)

The melting point is 1,222°F. and the flow point 1,416°F. The flux used was Lynco type H containing 10 per cent hydrazine. Coils of the solder were placed at intervals on the rustless alloy, melted with an air-gas orthodontic blowpipe, and air quenched.

The microscopic sections were prepared by mounting the samples in acrylic resin, followed by hand polishing on various grades of carborundum

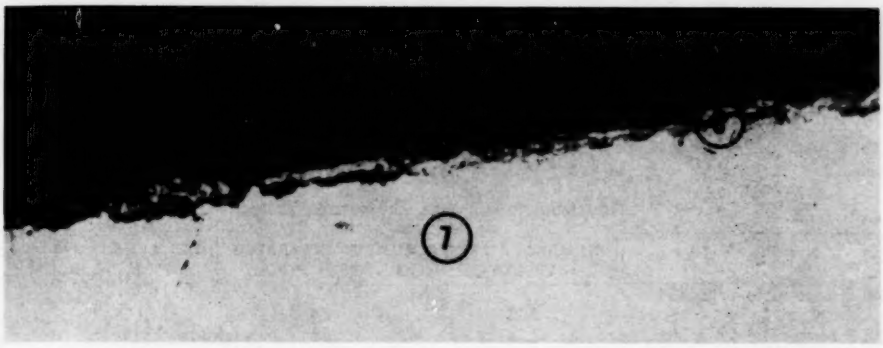

Fig. 3.—18-8 austenitic steel type 304 wire (7), gold plated for 5 minutes, soldered with silver solder (8), passivated for 20 minutes, mounted, polished, and etched with potassium cyanide. The gold plate appears to be clearly visible, porous, and with some areas separated from the steel. (Orig. mag. ×870.)

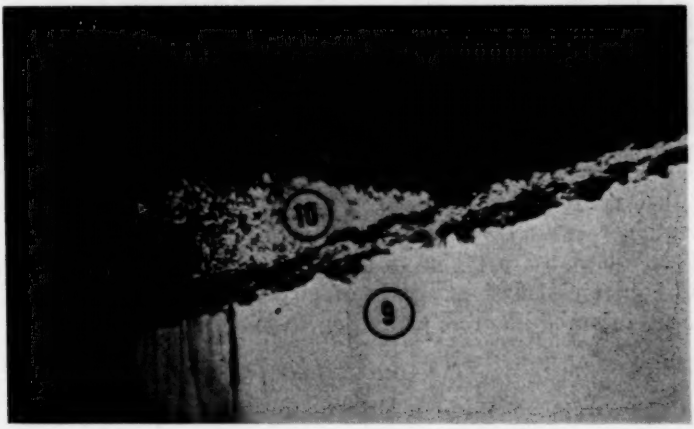

Fig. 4.—80 nickel–20 chromium (9) which was nickel flashed for 5 seconds prior to gold plating for 5 minutes. The wire was then soldered with silver solder (10), mounted, polished, then etched with potassium cyanide. (Orig. mag. ×870.)

and emery abrasive paper. This was followed by polishing on a metallurgical polishing wheel with diamond abrasive, finishing up with a quarter-micron size (Figs. 1-4).

On 18-8 austenitic steel type 304, 255 joints were made. On 17-7PH austenitic steel, 207 joints were made. On 80 nickel-20 chromium, 151 joints were made. In all, a total of 613 joints were studied in this particular series.

In the accelerated crevice corrosion test there were 244 joints of 18-8 austenitic steel type 304 of which 122 were plated as in *a*; 168 joints of 17-7PH austenitic steel of which 78 were plated as in *b* or *c*; 148 joints of 80 nickel-20 chromium of which 70 were plated as in *d*. These were immersed in a solution made up of 8 Gm. ferric sulfate (anhyd), 4 c.c. hydrofluoric acid, 96 c.c. water.

DATA

In all, a total of 560 joints were subjected to the accelerated crevice corrosion test. The results are given in Table III. It would appear that a significantly higher percentage of the joints of both the plated 18-8 and the plated 17-7PH austenitic steels were separated as compared with the unplated samples.

TABLE III

ACCELERATED CREVICE CORROSION TEST

	NUMBER JOINTS TESTED	NUMBER SEPARATED AFTER 24 HOURS	PER CENT SEPARATED AFTER 24 HOURS
18-8 austenitic type 304 plated *a*	122	28	23.0
18-8 austenitic type 304	122	23	18.9
17-7PH austenitic plated *b* or *c*	78	37	47.4
17-7PH austenitic	90	15	16.7
80 nickel-20 chromium plated *d*	70	0	0
80 nickel-20 chromium	78	2	2.6
Totals	560	105	18.8%

The unplated 17-7PH austenitic steel appeared only slightly less susceptible to crevice corrosion than the unplated 18-8 austenitic steel type 304 under the particular set of conditions imposed by this experiment. The plated 17-7PH austenitic steel, on the other hand, appeared to be more susceptible to crevice corrosion than the plated 18-8 austenitic steel type 304. A significant difference in the treatment of the two metals was that the 17-7PH austenitic steel had been nickel flash-plated previous to gold plating. The 80 nickel-20 chromium which had been nickel flash-plated in exactly the same manner as the 17-7PH austenitic steel showed no gross separation of joints in this test.

CONCLUSIONS

From the results of the accelerated crevice corrosion test and from the examination of the photomicrographs of the sections, it would appear that the reported procedure for electrodeposition of a thin layer of gold on 18-8 or 17-7PH austenitic steel prior to soldering does not ensure a crevice corrosion resistant joint.

It would appear, in confirmation of clinical findings, that 80 nickel-20 chromium is substantially more resistant to the accelerated crevice corrosion test than the other rustless alloys tested, particularly when gold is flash-plated to the 80 nickel-20 chromium.